COLORIMETRIC METHODS OF ANALYSIS

Including Some Turbidimetric and Nephelometric Methods

By

FOSTER DEE SNELL, Ph.D.

and

CORNELIA T. SNELL, Ph.D.

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PREFACE TO THE THIRD EDITION

In 1946, 23 per cent of all analytical papers published were in the field of colorimetry—many of them photoelectric—which indicates the trend.[1] In a majority of cases, inclusion of preparation of the sample with each specific method creates undue multiplicity of detail. Therefore, with rare exceptions, after a brief introduction to each chapter the methods of preparation of samples are associated in the following general sequence: metals, minerals, liquid samples, solid organic samples. The types of samples are necessarily limited to published work, and, for new types, an analogy to those available must often be sought.

Following that, one or more methods of preparation of standards apply to all or nearly all methods. The procedures which then follow are in approximate order of their importance but with occasional deviations to permit juxtaposition of related methods.

The question of critical selection of procedures necessarily arises at this point. When authors are highly critical, a book is correspondingly highly personalized and noninclusive. When authors are uncritical, a book becomes a mere collection of methods. The middle-of-the-road position selected here is rather inclusive but gives methods only briefly if they appear to be unimportant. In general, fewer than half of the available methods are given in detail. It must not be overlooked that different conditions require radically different methods. To take copper as an example; when concerned with traces, the diethyldithiocarbamate method is to be preferred. For somewhat larger amounts the ammonia method is suitable, particularly if maximum accuracy is not required, and over a broader range the bromide method is definitely suitable. A choice from a series of other methods may be made, according to the availability of the necessary reagent, or perhaps according to the type of instrument available.

The more important methods receive mention in an introductory portion of each chapter, designed to give general over-all familiarity with the chapter's content. Many chapters conclude with a "Miscellaneous" topic which contains in brief form, or as a generalized statement, methods which have not been discussed in the literature in the past ten years,

[1] Frederick C. Strong, *Anal. Chem.* 19, 968-70 (1947).

methods believed somewhat unsatisfactory, and methods not believed to be adequately established in chemical literature.

In order to avoid endless repetition it is assumed that the operator will make sure that reagents are free from the test substance. Thus a procedure for copper will not say to add "copper-free distilled water," but merely water. The sequence of chapters adopted conforms in general to the familiar qualitative system. This is preferred because it usually results in methods of similar derivation falling in close proximity, such as lead near mercury, aluminum near iron, silica near phosphorus.

Our objective is to supply the worker in colorimetry with a selected collection of available methods which can be applied with more or less standard colorimetric instruments. If the book saves times in avoiding the necessity of reference to a complete library, it will have served one purpose. If it helps in the selection of methods, it will have served another. As in the earlier volume, some of the older references have been omitted and can be found in the previous edition.

Foster Dee Snell

Cornelia T. Snell

New York, N. Y.
March 1949

CONTENTS

ILLUSTRATIONS

CHAPTER 1

LEAD

Work in the period since publication of the last edition has been largely devoted to refinement of the dithizone method, with secondary attention to the sulfide method. As usual, the main problem is in the separation of interfering metals. Several minor methods are given. The determination of amounts of lead measurable in parts per million is important in control of the processing or manufacture of foods, cosmetics, and many other products. The importance is greatly increased by the cumulatively poisonous nature of lead.

In general, when the amount of lead exceeds 0.001 mg., either the dithizone method or the spectrographic method may be used with equal reliability. For smaller amounts the spectrographic method is superior. The carbazide method is applicable for samples containing substantial amounts of lead so that a loss of 0.07 mg. inherent in the method is not significant.[1]

Dithizone Methods

The reagent diphenylthiocarbazone[2] or phenylazothionoformic acid, usually called dithizone, gives a series of brilliantly colored complexes with gold, platinum, palladium, silver, mercury, stannous, bismuth, copper, zinc, cobalt, nickel, lead, thallium, and cadmium ions. Because of its wide applicability the subject of lead determination is preceded at this point by a general discussion of dithizone determinations which is of general applicability and should be referred to for other dithizone methods.

The various complexes can usually be separated to give the color due to a single metal or the dithizone equivalent to it. Many are extracted into organic solvents. The most useful range is 0.001-0.2 mg. with errors of 1-5 per cent. The relation of the metals when converted to

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[1] Jacob Cholak, Donald M. Hubbard, Robert R. McNary and Robert V. Story, *Ind. Eng. Chem., Anal. Ed.* 9, 488-90 (1937).

[2] Hellmut Fischer, *Angew. Chem.* 46, 442-50 (1933); *ibid.* 47, 685-92 (1934); *ibid.* 50, 919-32 (1937).

dithizonates is well brought out by the curves in Figure 1.[3] The majority of conditions for separation are embodied in it.

Inorganic samples are usually merely dissolved in acid. Organic samples may be prepared by wet or dry ashing but are often extracted without such ashing, treatment with nitric acid serving as a substitute. Separation of interfering metals is often important.

The reagent exists in keto and enol forms, the colored compounds being usually formed with the keto form. Although the reagent is insoluble in water, it is soluble in ammonium hydroxide and in many organic solvents. Carbon tetrachloride and chloroform are ordinarily used. Mild oxidizing agents convert it to diphenylthiocarbazone which is yellow and does not react with metals. This may be reduced to

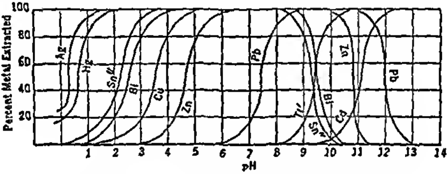

FIG. 1

Provisional Equilibrium Curves of Some Metal Dithizonates in Chloroform

dithizone by treatment with hydroxylamine hydrochloride or by sulfites, and oxidation may be prevented by them. Stronger oxidizing agents make more drastic, and irreversible, changes. A solution in carbon tetrachloride or chloroform is stable if kept cool and protected from light. A 0.64 per cent solution of sulfur dioxide floating on a dithizone solution in an organic solvent is effective for protection at 0° in the dark.

The solutions of the reagent ordinarily used are colored an intense green. The reagent is, and the complexes usually are, more soluble in chloroform than in carbon tetrachloride They are customarily formed by shaking an aqueous solution of the metallic ion at a controlled pH with dithizone in chloroform or carbon tetrachloride. The green color of the excess reagent in chloroform modifies the color of the metallic dithizonate. Mixed colors are not produced from alkaline aqueous solutions with carbon tetrachloride because the excess of dithizone is less and it is largely dissolved in the aqueous phase.

[3] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66 72 (1939).

Dithizone Reagent. The reagent often contains a yellow oxidation product from which it is separated by a purification procedure.[4] A grade is available from the Eastman Kodak Company which does not require such purification. As one technic, dissolve about 1 gram of the commercial grade in 50-75 ml. of chloroform. Filter if insoluble material remains. Extract with four 100-ml. portions of 1:100 redistilled ammonium hydroxide. The dithizone passes into the aqueous phase to give an orange solution. Discard the chloroform layer. Filter the combined aqueous extracts through cotton into a large separatory funnel. Add 1:1 hydrochloric acid until acid to litmus. The dithizone will precipitate. Extract this dithizone with three 20-ml. portions of chloroform. Wash these combined extracts with three portions of water, and discard the aqueous layer and washings.

Evaporate the chloroform solution of dithizone on a steam bath and heat for an hour *in vacuo* at not over 50°. Store the dry reagent in a tightly stoppered bottle. Make up a stock reagent containing 0.1 gram of dithizone per 100 ml. of chloroform, or 1 mg. per ml., and dilute it as necessary with chloroform to obtain the desired concentration. Refrigerated standard dithizone solutions[5] in chloroform will keep for months in darkness in glass-stoppered Pyrex containers. Whenever shaken with water, about 0.5 per cent of alcohol should be added to the chloroform as a preservative. It should be added to the chloroform accumulated for reclaiming, to the receiver for distillation, and after washing with sulfuric acid.

As a solution of the reagent in carbon tetrachloride, dissolve 0.5 gram of commercial dithizone in about 500 ml. of the solvent, and filter into a 5000-ml. separatory funnel containing 2-3 liters of 1:700 ammonium hydroxide. Shake well to extract the dithizone into the aqueous phase. Remove the carbon tetrachloride and run it through the paper to dissolve dithizone remaining, using the same separatory funnel as receiver. Repeat until no residue remains on the paper. Finally, after extraction, discard the carbon tetrachloride. Extract the ammoniacal solution with successive 50-ml. portions of carbon tetrachloride until no pink is shown in the extract. Add 500 ml. of carbon tetrachloride, and 1:1 hydrochloric acid until the aqueous layer is definitely acid. Shake until the dithizone goes into the carbon tetrachloride layer. If necessary use more carbon tetrachloride. Finally, dilute the extract to 1 liter with

[4] Isamu Namata and Danzi Matukawa, *J. Biochem.* (Japan) 30, 395-9 (1939).

[5] Karl Bambach, *Ind. Eng. Chem., Anal. Ed.* 11, 400-3 (1939); Karl Bambach and R. E. Burkey, *ibid.* 14, 904-7 (1942).

carbon tetrachloride and store in a glass-stoppered bottle in a cold, dark place.[6]

Other Reagents. The wide distribution of lead, and to a lesser extent of other ions determined with dithizone, necessitates careful attention to all reagents used. The following is illustrative of the amounts of lead which may be present in reagent-grade chemicals.[7]

	Mg. per liter (ppm.)
Double-distilled water	0.002
Concentrated nitric acid	0.01-0.02
Concentrated hydrochloric acid	0.01-0.03
Concentrated sulfuric acid	0.8
Potassium cyanide, 10 per cent solution	0.08

Dithizone is not only a reagent for the determination of lead but for purification of some reagents and for isolation of some samples.

To test reagents for lead take 15-20 ml. of concentrated solutions or 10-15 grams of solid dissolved in redistilled water. Add sufficient lead-free citric acid to prevent precipitation of such substances as iron and aluminum hydroxides and phosphates by ammonia. Add 1:1 ammonium hydroxide until the solution is definitely alkaline, and 2-3 ml. of 10 per cent potassium hydroxide solution. Shake the solution with about 5 ml. of dithizone solution containing about 10 mg. per liter of chloroform. If the lower layer is red, it definitely indicates contamination. If green, remove the layer and extract with 1:100 ammonium hydroxide to which a drop of 1 per cent potassium cyanide solution has been added. If the organic solvent is then colorless rather than pink, the test for lead is negative and the reagent may be used without purification.

Reagents must often be redistilled. Before use of a new glass still, steam it out with nitric acid vapors to remove lead sorbed on the surface and follow by steam to remove acid. Wash new glassware with hot 10 per cent sodium hydroxide solution or chromic-sulfuric acid cleaning solution, followed by hot 1:1 nitric acid, and copious rinsing in water.

Redistill water from an all-glass still and store in Pyrex bottles which have been washed with nitric acid. As another technic to prepare lead-free water,[8] dissolve 1 gram of disodium phosphate in 3 liters of ordinary distilled water in Pyrex. Add 1 gram of precipitated calcium

[6] P. A. Clifford, *J. Assoc. Official Agr. Chem.* **21**, 212-18 (1938).
[7] Donald M. Hubbard, *Ind. Eng. Chem., Anal. Ed.* **9**, 493-5 (1937).
[8] Thomas D. Gray, *ibid.* **14**, 109-14 (1942).

carbonate and stir thoroughly. Let the suspension settle overnight and filter into glass-stoppered bottles. Check each bottle for lead by testing.

Distillation is also appropriate for purification of ammonium hydoxide, nitric acid, hydrochloric acid, chloroform, etc.

Another technic for hydrochloric acid is to add concentrated sulfuric acid to the C.P. grade and absorb the evolved acid vapors in triple-distilled water [9] until the concentration is 0.2 *N*. For ammonium hydroxide dilute 50 ml. of C.P. grade to 1 liter and wash with 10 ml. portions of dithizone containing 0.2 gram per liter in chloroform until the past portion shows no extraction of color. Now wash the ammonium hydroxide with 10 ml. portions of chloroform to remove entrained dithizone until the last washing is colorless. This method of purification is also applicable to salt solutions.

To purify such solutions of salts as sodium or ammonium acetate, add 5-10 mg. of copper sulfate to serve as collecter, adjust the pH to 3.0-3.5 with bromophenol blue as indicator, and saturate the solution with hydrogen sulfide. Filter the precipitated mixed sulfides, preferably through an inorganic filter, and boil for 20 minutes to drive off hydrogen sulfide. If necessary, filter again. Store in Pyrex bottles.

To delead ammonium citrate solution, render it alkaline to phenol red and extract with a dithizone solution containing 10 mg. per liter of chloroform. For potassium cyanide, similarly extract a saturated aqueous solution without prior adjustment of alkalinity. Then extract the dithizone from the aqueous phase with chloroform before diluting to the final concentration.

To purify hydroxylamine hydrochloride, dissolve 20 grams in water and dilute to about 65 ml. Add *m*-cresol purple indicator solution and then concentrated ammonium hydroxide until the indicator is yellow. Add sufficient 4 per cent diethyldithiocarbamate to precipitate reactive metals present and leave an excess. Extract the complexes and excess reagent with chloroform. The end point is reached when the chloroform extract, on shaking with a solution of a copper salt, shows no yellow color. Add hydrochloric acid to the hydroxylamine hydrochloride solution until it turns pink and dilute to 100 ml. with water.

Technics. The three general types of dithizone technics are monocolor methods, mixed color methods, and titrametric extraction. They do not all apply to all ions; but often, as in the case of lead, they do.

In the monocolor methods the ion is extracted from aqueous solu-

[9] J. Cholak, D. M. Hubbard and R. E. Burkey, *ibid.* 15, 754-9 (1943).

tion by an organic solvent solution of dithizone. At that stage a mixed color solution results due to the metallic dithizonate of one color and the reagent of another. Extraction of excess reagent can be carried out with variable success according to the ion. Usually the metallic dithizonate shows an appreciable loss in such extraction so that low results tend to be obtained. This can only be compensated by exactly similar treatment of the standard. They are usually accurate to 0.001 mg.

A variation is to convert the metallic dithizonate to dithizone in the solvent phase for reading, after excess reagent has been removed. Some dithizonates in organic solvent are easily transferred to the aqueous phase by shaking with very dilute acid; others require stronger acid; some are not quantitatively so transferable. Some are decomposed by strong alkali with the metal appearing in the aqueous phase as the hydroxide. The solvent has an effect, lead dithizonate in chloroform being decomposed at pH 11.0 as compared with pH 10.0 for the carbon tetrachloride solution.[10]

Cyanide forms a more stable complex with ions other than lead, bismuth, stannous and thallium than does dithizone. Therefora only these four dithizone complexes will be extracted from cyanide solutions. Potassium iodide and sodium thiosulfate form complexes in acid solution with many of the ions, but these complexes are decomposed in alkaline solution.[11] Diethyldithiocarbamate has also been used for forming complexes.[12] After separation of metallic ions by dithizone the ion may be liberated by oxidation and determined by another method.

In two-color methods the excess of dithizone is allowed to modify the color of the metal dithizonate. This gives a gradation of color suitable for the series-of-standards methods. Alternatively the absorption may be determined with suitable color filters and checked by reading the excess of dithizone with another color filter. The color may be matched by duplication. Ordinarily the colors obey Beer's law.

Loss of lead is avoided in two-color methods by extraction with excess reagent in solvent. The excess reagent partially passes into the aqueous layer, partially remains in the solvent. At pH 9.5 the amount of reagent in the solvent layer can be a source of serious error, since

[10] Herbert Müller, *Z. anal Chem.* **113**, 161-82 (1938).

[11] W. O. Winkler, *J. Assoc. Official Agr. Chem.* **18**, 638-44 (1935); Hellmut Fischer and Grete Leopoldi, *Z. anal. Chem.* **107**, 241-69 (1937); E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* **29**, 464-9 (1937); W. O. Winkler, *J. Assoc. Official Agr. Chem.* **21**, 220-8 (1938).

[12] R. A. Caughey, E. B. Holland and W. S. Ritchie, *J. Assoc. Official Agr. Chem.* **21**, 204-7 (1938).

there is some light absorption by the reagent in the same region as that in which lead dithizonate is read. Therefore, under those conditions, it is necessary to approximate a definite ratio of lead to the reagent. Thus the amount of lead has to be known approximately to determine the amount of reagent to use. This is simplified if extraction is at a pH carefully adjusted to 11.5. Then the excess dithizone goes substantially quantitatively into the aqueous phase.

For titrametric extraction methods, the high density of the solvents permits extraction of a sample with several successive fractions of reagent. Control of the concentration of dithizone in the organic solvent phase is necessary for some technics. Generally the more noble a metal the lower the pH which permits its optimum extraction. In some cases extraction with controlled amounts of dithizone is successive, as of copper, then mercury, then lead at pH 4, provided one is not in very large excess. Curves of the equilibria between different metals at different pH values would be complex and have not been determined.

The color may be read or the dithizonate converted to dithizone for reading its color. Such readings are usually sensitive to 0.001 mg.

The tedious titrametric extraction methods for lead depend on successive extractions at pH 7.5-8.0 from aqueous solution by small increments of standardized dithizone solution. Care in getting the end point is necessary.

Reclaiming of Chloroform. It is usual to recover the chloroform used as solvent for the reagent. Acceptable technics follow. Methods of recovery of carbon tetrachloride in a form suitable for this reagent have not been devised.[13]

Remove any aqueous layer [14] from the accumulated residues. Wash the chloroform with 5-10 per cent of its own volume of concentrated sulfuric acid to remove organic impurities. Repeat until the chloroform layer is colorless. Withdraw the acid as completely as possible and add hydrated lime in excess to neutralize any sulfuric acid left and any acid from decomposition during later distillation. Transfer with the suspended lime to a flask and distill. Add 1-1.5 per cent of alcohol to the distillate to serve as a preservative. This meets USP standards and is suitable for general reagent use.

Alternatively, neutralize a 0.5 per cent solution of hydroxylamine hydrochloride with 1:100 ammonium hydroxide to phenol red, about

[13] S. L. Morrison and Harriet L. Paige, *Ind. Eng. Chem., Anal. Ed.* 18, 211-13 (1946).

[14] Donald A. Biddle, *ibid.* 8, 99 (1936).

pH 7.5. Shake 1 liter of chloroform with 100 ml. of this. Remove traces of water from the chloroform by filtration through cotton.

SAMPLES

Magnesium Alloys.[15] Weigh out a sample which is expected to contain 0.1-0.7 mg. of lead. Add 30 ml. of water, then slowly add 20 ml. of 1:1 hydrochloric acid per gram to decompose the sample in the cold. When reaction ceases, heat to boiling and dilute to about 200 ml.

Bismuth, thallium, indium and stannous ions are assumed to be absent in these alloys for the technic outlined. If necessary, remove bismuth by dithizone prior to determination as the dithizonate (page 37). If necessary, oxidize tin to the stannic form by adding 1 ml. of concentrated nitric acid and boiling. Then add hydroxylamine hydrochloride in slight excess, and boil to reduce the other constituents. Transfer to a 500-ml. volumetric flask, dilute to volume, and mix. Use 10-ml. aliquots of this solution as samples, the dithizone method being recommended. Titrate another 10 ml. aliquot with 1:9 ammonium hydroxide until alkaline to methyl red, and add that amount to the sample.

Zinc. Dissolve a 20-gram sample in 200 ml. of 1:1 nitric acid, and evaporate the excess acid until the residue is sirupy. Let cool and add about 10 ml. of 1:50 nitric acid. Dissolve by warming and add 1 gram of urea to destroy excess nitric acid. Boil, let cool, and dilute to 100 ml. without filtering. Use an aliqnot as sample by the dithizone method. When developed there should be sufficient ammonium hydroxide present to redissolve any zinc hydroxide.

Steels.[16] Dissolve a 1.0-gram sample in 25 ml. of a 1:1 mixture of concentrated hydrochloric and nitric acids. Add 25 ml. of 70 per cent perchloric acid and a few drops of 48 per cent hydrofluoric acid. Heat to strong fumes of perchloric acid to oxidize chromium. When cool add 150 ml. of distilled water and then add 1:1 ammonium hydroxide with stirring until the localized precipitate of ferric hydroxide just dissolves on further stirring. Saturate the solution with hydrogen sulfide, add 2 drops of 1:1 ammonium hydroxide, and continue to pass in hydrogen sulfide for 3 minutes longer.

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15 "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 328-30. American Society for Testing Materials, Philadelphia, Pa.

16 Lewis G. Bricker and Kenneth L. Proctor, *Ind. Eng. Chem., Anal Ed.* **17**, 511-12 (1945).

Filter and wash with a saturated aqueous solution of hydrogen sulfide. Ignite the precipitate and cool. Dissolve the residue so far as possible in 10 ml. of hot 1:1 nitric acid. After trituration of the particles with a glass rod and digestion for a few minutes, filter, wash with 1:100 nitric acid, and discard the paper and residue. Use the filtrate as sample and in application of the dithizone method, add 5 ml. of 50 per cent citric acid. Direct extraction of lead by dithizone from steel solutions is not feasible.

Copper.[17] Wash a 50-gram sample with hot concentrated hydrochloric acid and then with distilled water. Dissolve in 200 ml. of concentrated nitric acid. Boil off the brown fumes and add 1200 ml. of water. Add 350 ml. of concentrated ammonium hydroxide and 2 ml. of 50 per cent lead-free disodium phosphate. Add 50 ml. of an ammonium carbonate solution prepared by passing carbon dioxide into 1:2 ammonium hydroxide until a precipitate starts to form. Mix well and add 25 ml. of 20 per cent lead-free calcium chloride solution with stirring. Let stand overnight and filter on an inorganic filter. Dissolve the precipitate in 25 ml. of concentrated hydrochloric acid. Neutralize with 1:1 ammonium hydroxide, and slightly acidify with concentrated hydrochloric acid. Saturate with hydrogen sulfide and filter. Wash on the filter with 1:50 hydrochloric acid saturated with hydrogen sulfide. Dissolve tho sulfides in a suitable volume of hot concentrated nitric acid. Evaporate to crystals on a water bath. Cool and take up with 10 ml. of ammonium acetate solution made by mixing 1 volume of 1:1 ammonium hydroxide with 2 volumes of 35 per cent acetic acid solution. Dilute to a suitable volume and use all or an aliquot as sample.

Brass.[18] Dissolve a 1.5-gram sample containing 0.03-2.5 per cent of lead in 15 ml. of 1:1 nitric acid. When solution is complete, heat vigorously until evaporated to small volume. Tin will be present as a precipitate of metastannic acid. Filter and wash. Dilute to about 50 ml. Add a saturated solution of sodium carbonote until reduced to neutrality. There will be some precipitation. Add glacial acetic acid with stirring until solution is complete, then 5 ml. in excess. Add 20 ml. of half-saturated potassium bichromate solution to precipitate the lead. Heat to boiling for about 5 minutes, then filter. Wash the filter with 1:20 acetic acid, then with water until the filtrate shows a negative

[17] Bartholow Park and E. J. Lewis, *ibid.* 7, 182-3 (1935).
[18] R. E. Oughtred, *Analyst* 70, 253-4 (1945).

reaction for chromate. Dissolve the precipitate from the paper with 1:1 nitric acid and dilute to a known volume. The chromate color may be read as a method of indirect determination, or this sample may be used for development of color with *s*-diphenylcarbazide.

Silicate Rocks.[19] Weigh a 0.25-gram 100-mesh sample into a platinum dish. Add 0.5 ml. of 70 per cent perchloric acid, 3 ml. of water, and 3 ml. of 48 per cent hydrofluoric acid. Let this boil gently on a hot plate, stirring occasionally with a platinum wire until crystallization begins, remove the wire, and evaporate to dryness. Let cool somewhat, and add 0.5 ml. of 70 per cent perchloric acid and 2 ml. of water. Again evaporate to dryness, being sure to expel excess perchloric acid. Add 7 ml. of 1:6 hydrochloric acid to the cooled residue and warm, if necessary, until the soluble matter is in solution. Add 5 ml. of 10 per cent sodium citrate solution containing 0.5 ml. of concentrated ammonium hydroxide per 100 ml. Cool to room temperature, add 1:1 ammonium hydroxide dropwise until the solution is alkaline to litmus, and 0.5 ml. excess. If the solution is turbid at this stage, let it stand for at least 10 minutes.

Filter the solution and transfer any residue to the paper. Wash the filter three times with 1-ml. portions of water, each containing a drop of concentrated ammonium hydroxide and a drop of the 10 per cent sodium citrate solution. If turbidity develops in the filtrate on standing, refilter. Reserve the filtrate as solution 1.

Ignite the paper with the residue in a platinum crucible at a low temperature. Let cool, add 0.15 gram of sodium carbonate, and fuse. When cooled, add about 3 ml. of water and warm to dissolve the soluble portion. Filter through a small paper, leaving the bulk of the residue in the crucible, and wash the filter with 8-10 ml. of water. Reserve this filtrate as solution 2.

Transfer the paper and residue to the same crucible and ignite. Let cool and add 1 ml. of 48 per cent hydrofluoric acid and 2 drops of 70 per cent perchloric acid. Evaporate to dryness and expel the excess perchloric acid. Let cool and add a drop of 70 per cent perchloric acid and a few drops of water. Evaporate again and let cool. Add 6 ml. of 1:6 hydrochloric acid to the residue. Heat until all solubles are extracted and filter. Wash on the filter, the combined solution and washings being solution 3. Unless extreme accuracy is required, discard the resi-

[19] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* **9**, 464-9 (1937).

due. If essential, fuse the residue according to the preceding paragraph and combine with solution 2.

To isolate the lead, copper, and zinc, extract solution 1 with 5 ml. of 0.01 per cent dithizone in carbon tetrachloride. After separation, unless it is distinctly green, make another extraction. The final extract must show the green of excess dithizone. To solution 2, add 0.5 ml. of 10 per cent ammonium citrate solution and 1:1 hydrochloric acid until greenish to thymol blue. Extract with 2-ml. portions of the dithizone solution. Extract solution 3 with 1-ml. portions of the dithizone solution.

Combine the extracts and wash with about 3 ml. of water. Extract the washings with 1 ml. of dithizone solution. Add this dithizone solution to the extracts and discard the washings.

To separate the copper, zinc, and lead, shake the combined extracts with 10 ml. of 1:500 hydrochloric acid for a minute. If the solvent layer is not now distinctly green, add 2 ml. more of reagent solution, and shake again. Withdraw the carbon tetrachloride layer and shake it with another 10 ml. of 1:500 hydrochloric acid. Combine the acid extracts which contain the lead and zinc. Wash with 2 ml. of carbon tetrachloride and add the washings to the solvent extract. Reserve the combined solvent solution for determination of copper. Dilute the acid solutions to 25 ml. for the use of aliquots for lead and zinc.

Solutions High in Iron.[20] With less than 100 ppm. of lead present, iron can be removed by extraction as the thiocyanate with organic solvents. At 12°, lead thiocyanate is practically insoluble in the extraction medium. The method does not provide for interference by bismuth.

Add 5 ml. of 1:5 nitric acid to the sample of 30 ml. of solution in a separatory funnel. Add 5 ml. of a saturated aqueous solution of ammonium thiocyanate and mix. Add 15 ml. of amyl alcohol and 15 ml. of ether. Cool to 12° and shake. Draw off the aqueous layer and extract with fresh organic solvent. Use the aqueous layer as sample after aliquoting if necessary.

Magnesium Carbonate.[21] Dissolve 2 grams of sample in 10 ml. of 1:1 nitric acid. Heat to boiling to drive off carbon dioxide. Dilute to about 75 ml., add 3 ml. of a 40 per cent solution of ammonium citrate, then add 1:1 ammonium hydroxide until alkaline to phenol red. Add 0.5 ml. of a 20 per cent solution of hydroxylamine hydrochloride and

[20] J. Hubert Hamence, *Analyst* 57, 622-6 (1932).

[21] Joseph Schultz and Melvin A. Goldberg, *Ind. Eng. Chem., Anal. Ed.* 15, 155-8 (1943).

1 ml. of a 10 per ceut solution of potassium cyanide. The preparation of sample does not provide for possible presence of bismuth or thallium. It is designed for dithizone extraction.

Zinc Oxide. Dissolve 0.1 gram of sample in 1 ml. of 1:1 nitric acid. Complete as for magnesium carbonate starting with "Dilute to about 75 ml. . . .".

Zinc Stearate. Ash 0.5 gram of sample in silica in a muffle furnace at 475-500°. This usually reqnires 15-20 minutes. Wet the cooled ash with 3 drops of concentrated nitric acid, dry over a free flame, and again asb in the muffle for 15-20 minutes. Repeat until a white ash is obtained. Dissolve the ash in 1 ml. of 1:1 nitric acid and complete as for magnesium carbonate starting at "Dilnte to about 75 ml. . . .".

Ferric Oxides. Dissolve 0.1 gram in 2 ml. of concentrated hydrochloric acid by boiling. Add 3 drops of concentrated nitric acid and boil to remove chlorine and oxides of nitrogen. Add water if necessary to avoid going to dryness. Dilute to about 75 ml. Add 5 ml. of a 40 per cent solution of ammonium citrate and 5 ml. of 20 per cent hydroxylamine hydrochloride solution. Make alkaline to phenol red with 1:1 ammonium hydroxide and add 4 ml. in excess. Cool and add 6 ml. of 10 per cent potassium cyanide solution. This is designed for the dithizone method of determination.

Calcium Carbonate. Dissolve a 5-gram sample in 15 ml. of 1:1 nitric acid. Heat to boiling to drive off carbon dioxide and cool. Add 5 ml. of 40 per cent ammonium citrate solution and 5 ml. of a 0.16 per cent solution of hydrated copper sulfate. Add 1:1 ammonium hydroxide until the pH is 3.0-3.4 as indicated by bromophenol blue. Saturate the solution with hydrogen sulfide. Prepare a wash solution of 3 per cent sodium sulfate, adjusted to pH 3.0-3.4 with sulfuric acid and saturated with hydrogen sulfide. Filter the lead sulfide and wash 3 times with the wash solution.

Dissolve the precipitate from the paper with 4 ml. of hot 1:1 nitric acid, and use the original precipitation flask as receiver. Wash the paper thoroughly with water. Boil the combined filtrates to eliminate hydrogen sulfide. Add 3 ml. of 40 per cent ammonium citrate solution, make alkaline to phenol red with 1:1 ammonium hydroxide, and add 1 ml. in excess. Add 0.5 ml. of 20 per cent hydroxylamine hydrochloride solu-

tion and 1 ml. of 10 per cent potassium cyanide solution. This is designed for determination by dithizone extraction.

Titanium Dioxide. *Acid-extractable Lead.* Add 0.5 gram to 10 ml. of 1:1 nitric acid in a 15-ml. graduated centrifuge tube. Digest for 1 hour in a boiling water bath with frequent agitation. Centrifuge and decant the clear layer. Add 10 ml. of 1:4 nitric acid, mix well, and digest in a boiling water bath for 15 minutes. Centrifuge and decant. Add 3 ml. of 40 per cent ammonium citrate solution to the combined decantates, make alkaline to phenol red with 1:1 ammonium hydroxide, and add 1 ml. in excess. Add 0.5 ml. of 20 per cent hydroxylamine hydrochloride solution and 1 ml. of 10 per cent potassium cyanide solution for dithizone extraction.

Total Lead. Fuse 0.2 gram of titanium dioxide with 2.0 grams of potassium bisulfate in a covered crucible, only until a clear melt is obtained. When the melt has cooled as a thin layer, add 4 ml. of 40 per cent ammonium citrate solution and 6 ml. of water. Heat carefully until the melt dissolves. Transfer to a flask with water and complete as for calcium carbonate, starting at "Add 3 ml. of 40 per cent ammonium citrate solution. . . ." Even small amounts of titanium will prevent the extraction of lead by dithizone. Very complete washing of the sulfide precipitate is necessary but a double precipitation of sulfides is not.

Talc. *Acid-extractable Lead.* Add 1 gram of sample to 10 ml. of 1:1 nitric acid in a 15-ml. graduated centrifuge tube. Complete as for titanium dioxide, starting at "Digest for 1 hour. . . ."

Total Lead. Put 6 ml. of 1:1 sulfuric acid and 3 ml. of 48 per cent hydrofluoric acid in a platinum crucible. Add 1 gram of sample, put in a sand bath at 200-250°, and heat until fumes of sulfur trioxide are given off. Add 1 ml. of 48 per cent hydrofluoric acid to the cooled crucible and repeat. Add 0.5 ml. of 48 per cent hydrofluoric acid and repeat. This time, fume for several minutes to be sure that all fluoride is driven off as it will otherwise be difficult to get a clear solution. When cool take up with water and complete as for calcium carbonate starting at "Add 3 ml. of 40 per cent ammonium citrate solution. . . ."

Kaolin. *Acid-extractable Lead.* Add 1 gram of sample to 10 ml. of 1:20 nitric acid in a 15-ml. graduated centrifuge tube. Complete as for titanium dioxide starting at "Digest for 1 hour . . ." but also use 1:20

nitric acid for the second extraction. Extraction of too much aluminum leads to unsatisfactory results.

Total Lead. Put 5 ml. of 1:1 sulfuric acid and 1.5 ml. of 48 per cent hydrofluoric acid in a platinum crucible. Add 0.5 gram of sample, place in a sand bath at 200-250°, and heat until fumes of sulfur trioxide are given off. Add 5 drops of concentrated nitric acid to the cooled crucible and again heat to sulfur trioxide fumes. Transfer to a flask with about 25 ml. of water and add 5 ml. of 40 per cent ammonium citrate solution. Heat to boiling for a few minutes until a clear solution is obtained. Cool and adjust the pH to 3.0-3.4 as indicated by phenol blue by adding 1:1 ammonium hydroxide. Add 5 ml. of 0.16 per cent solution of hydrated copper sulfate and complete as for calcium carbonate starting at "Saturate the solution with hydrogen sulfide." The separation of lead is to avoid interference by aluminum.

Barium Sulfate. *Acid-extractable Lead.* Add a 1-gram sample to 10 ml. of 1:1 nitric acid in a 15-ml. graduated centrifuge tube. Complete as for titanium dioxide starting at "Digest for 1 hour in a boiling water bath. . . ."

Total Lead. Mix 1 gram of sample, about 2 grams of sodium carbonate, and about 3 grams of potassium carbonate in a platinum crucible. Fuse to a clear, mobile liquid and let cool. Add a few ml. of water and heat over a flame to loosen the melt. Transfer with 20-25 ml. of water and boil until completely disintegrated. Filter while still hot and wash with hot 5 per cent sodium carbonate solution until the wash liquid gives no test for sulfate. Reserve this combined filtrate and washings, wash the precipitate twice with water, and discard these washings. Add 5 ml. of 40 per cent ammonium citrate solution, 0.5 ml. of 20 per cent hydroxylamine hydrochloride solution, and 1 ml. of 10 per cent potassium cyanide solution to the reserved solution.

Separately dissolve the residue from the filter in 8 ml. of 1:3 hydrochloric acid, and wash the paper thoroughly with water. Heat the solution and washings to boiling to eliminate carbon dioxide and let cool. Add 3 ml. of 40 per cent ammonium citrate solution, and then 1:1 ammonium hydroxide until alkaline to phenol red, and 1 ml. excess. Add 0.5 ml. of 20 per cent hydroxylamine hydrochloride solution and 1 ml. of 10 per cent potassium cyanide solntion. Determine lead separately in the two solutions by dithizone extraction.

Bismuth Subcarbonate.[22] The procedure for this bismuth salt is a general procedure for separation of lead from bismuth. Dissolve a 1-gram sample in a minimum amount of concentrated nitric acid by heating. The balance of the treatment is given as the method of separation of lead and bismuth (page 33).

The same reference gives about 30 methods of treatment of other pharmaceutical samples to prepare them for analysis. All are less complex than that cited.

Creta Praeparata.[23] This form of precipitated chalk is added to bread flour in England at the rate of about 1 ounce per 20 pounds of flour. To separate the lead, add 45 ml. of 1:5 hydrochloric acid to a 5-gram sample. Boil until solution is as complete as possible and filter. Return the residue to the original flask and boil with 5 ml. of concentrated hydrochloric acid. Filter, catching the solution with the previous filtrate, and wash the filter with about 10 ml. of hot water. Dilute to a known volume and use an aliquot of this extract as sample. The original method was to determine as colloidal sulfide.

Calcium Phosphate.[24] Dissolve a 1-gram sample in 10-15 ml. of 1:10 hydrochloric acid. Add 30 ml. of 50 per cent ammonium citrate solution. Add sufficient 1:1 ammonium hydroxide to render the solution distinctly alkaline and add 2 ml. of 2 per cent potassium cyanide solution. Complete by the sulfide method.

Commercial Phosphoric Acid. Weigh 100-400 grams of commercial phosphoric acid, depending on its concentration and purity, into a liter flask, and dilute with water.[25] Warm if necessary to make the solution clear, cool, and dilute to 1 liter. Transfer an aliquot containing 5-15 grams of acid to a 250-ml. beaker. Add 10 ml. of 25 per cent sulfuric acid and about 1 gram of a paste of calcium sulfate. Add sufficient 95 per cent ethanol to make a 70 per cent solution. When the precipitate begins to settle, filter and wash with 70 per cent ethanol. Transfer the filter paper and precipitate containing the lead to a small beaker, and add 50 ml. of ammoniacal ammonium acetate solution made by pouring 1 volume of 1:1 ammonium hydroxide into 2 volumes of 35 per cent acetic acid. Boil for a few minutes and decant through a filter.

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[22] Karl Bambach, *Ind. Eng. Chem., Anal. Ed.* **12**, 63-6 (1940).

[23] E. C. Dawson and A. Rees, *Analyst* **71**, 417-19 (1946).

[24] John R. Nicholls, *ibid.* **56**, 594-5 (1931).

[25] W. H. Ross, C. B. Durgin and R. M. Jones, *J. Ind. Eng. Chem.* **14**, 534 (1922).

Digest the residue with 2 fresh portions of ammonium acetate solution. The greater part of the calcium sulfate should have been dissolved. Cool the combined filtrates, acidify with acetic acid, dilute to a known volume, and use an aliquot as sample.

Baking Powder. Place 10 grams of sample in a Kjeldahl flask, washing down with a few ml. of water. Add 200 ml. of concentrated sulfuric acid for samples containing not over 2 grams of starch, and 5-10 ml. for each additional gram of starch. Add 5-10 grams of potassium bisulfate. Heat to boiling and digest until the carbonaceous matter is oxidized, adding a few ml. of concentrated nitric acid from time to time to hasten oxidation of carbon. Let cool, dilute, and evaporate in a casserole to complete removal of sulfur trioxide fumes.

To remove copper, iron, aluminum, and similar metals, extract the residue 5-6 times with portions of a cold solution containing 25 ml. of 5 per cent sulfuric acid and 5 ml. of 95 per cent ethanol. Prepare ammonium acetate solution by neutralizing concentrated ammonium hydroxide with glacial acetic acid and adding 5 per cent by volume of concentrated ammonium hydroxide in excess. Digest the undissolved residue containing lead sulfate with 50 ml. of ammonium acetate solution, heated to boiling. Extract 2-3 times, filter through the same paper that was used for the removal of iron, and wash with water. Evaporate the extract to about 40 ml. Cool and dilute to a known volume for use of aliquots.

Shaving Cream.[26] Dissolve or disperse a 10-gram sample in 50-75 ml. of hot water and add 15 ml. of concentrated nitric acid. Heat nearly to boiling, transfer the clear aqueous layer to a 100-ml. calibrated flask, and after cooling dilute to volume.

Water and Salt Solutions.[27] Add 1:1 hydrochloric acid to 500-2000 ml. of sample until distinctly acid and evaporate to about 75 ml. over an open flame. Add about 2 grams of ammonium chloride to hold magnesium in solution and help coagulate sulfides. Add 1:1 ammonium hydroxide to neutrality and 2 ml. in excess. Saturate with hydrogen sulfide and let stand for 2 hours. Add another 2 ml. of 1:1 ammonium

[26] Frank H. Buckwalter, *Proc. Sci. Sect. Toilet Goods Assoc.* 1943 4, No. 1, 22-4; *Soap, Perfumery, Cosmetics* 17, 521-2 (1944).

[27] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, pp. 636-9. Association of Official Agricultural Chemists, Washington, D. C. (1945).

hydroxide and again saturate with hydrogen sulfide. Boil for a few minutes and let the precipitate settle. Filter and wash the precipitate once with hot water. To the paper and filter add 30 ml. of 1:3 nitric acid and heat to boiling. Filter and wash with water until free from acid. This solution contains the iron, lead, copper, and zinc.

Add 5 ml. of 1:1 sulfuric acid and evaporate until copious fumes of sulfur trioxide are given off. Let cool, wash down the sides, and repeat the evaporation to copious fumes. Transfer to a beaker with about 10 ml. of water and add an equal volume of 95 per cent ethanol. After standing overnight filter the lead sulfate and wash with 50 per cent ethanol until free from iron. This filtrate contains iron, copper, and zinc and is for later use in their determination.

Digest the filter and lead sulfate with 40 ml. of 40 per cent ammonium acetate solution. Filter and wash the filter once with the ammonium acetate solution, then with water. Dilute the filtrate to a known volume and determine lead in an aliquot by the sulfide method. Due to the treatment only the addition of sulfide is necessary.

Alternatively,[28] take a volume of water which will yield 0.05-0.10 mg. of lead. Evaporate to a small volume in a Pyrex flask. Add 1 ml. each of concentrated sulfuric acid and 70 per cent perchloric acid. Continue to heat until all organic matter is destroyed and copious fumes are evolved. Take up in a small volume of water and use as sample by the dithizone method.

Plating Baths.[29] Use 5-20 ml. of the bath, as indicated by preliminary examination.

Urine.[30] Add 10 per cent by volume of concentrated nitric acid to 50 ml. or a multiple of that volume of urine in a silica or Pyrex evaporating dish. Evaporate to dryness on a hot plate or steam bath. Ignite in a muffle at 500°. Let cool and moisten the ash with concentrated nitric acid. Dry and ignite in the muffle to get a white ash. Dissolve the ash in the minimum feasible volume of 1:1 nitric acid and dilute to a known volume in order to take aliquots.

Transfer 15 ml. of 50 per cent ammonium citrate solution to a separatory funnel. Add an aliquot of the sample, 5 ml. of 10 per cent potassium cyanide solution, and 1 ml. of 20 per cent hydroxylamine hydro-

[28] Sidney L. Tompsett, *Analyst* 61, 591-7 (1936).
[29] D. Gardner Foulke, *Monthly Rev. Am. Electroplaters' Soc.* 31, 1103-8 (1944).
[30] Karl Bambach and Roland E. Burkey, *Ind. Eng. Chem., Anal. Ed.* 14, 904-7 (1942); cf. F. Morton, *Analyst* 61, 465-71 (1936).

chloride solution. Add distilled ammonium hydroxide until alkaline to phenol red. If the solution becomes cloudy during this treatment, add more ammonium citrate to redissolve calcinm phosphate.

Extract the solution with 10 ml. of a solution of 10 mg. of dithizone per liter of chloroform. Drain and extract with further 5-ml. portions until one remains green. Wash the combined extracts with 50 ml. of water. Wash the water with 5 ml. of chloroform, returning this to the chloroform extract. This wash should be green.

Dilute 9.1 ml. of concentrated nitric acid to approximately 500 ml. with double-distilled water in a 1-liter volumetric flask. Add bromothymol blue indicator and adjust the pH to 3.4 with 1:1 ammonium hydroxide. Add 25 ml. of 0.2 *M* acid potassium phthalate solution (Vol I, page 170) and 4.97 ml. of 0.2 *M* hydrochloric acid (Vol. I, page 172) and dilute to volume. This is a bnffer for pH 3.4.

Shake the solution containing the lead with 50 ml. of the buffer solution. Unless the chloroform layer returns to pure green there is bismuth present. If so, wash the buffer extract of lead with 5 ml. of dithizone reagent. Dilute the buffer solution of lead to a known volume and use all or an aliquot by the dithizone method.

Alternatively[31] make 200 ml. of urine in a centrifuge cup faintly acid to litmus. Add 3 ml. of 10 per cent calcium chloride solution and mix. Add 20 ml. of saturated ammonium oxalate solution to precipitate lead and calcium. Let stand for 1 hour and centrifuge. Decant the upper layer carefully and completely. Add 2 ml. of 60 per cent perchloric acid and digest over a low flame. Add 30 per cent hydrogen peroxide dropwise from time to time until the solution remains colorless. Let cool, take up in water, and dilute to about 50 ml. Pass sulfur dioxide through the solution and let stand for an hour.

If undue interference with lead is enconntered,[32] extract the lead from the ash solution with diethyl dithiocarbamate and ether. Evaporate the lead solution so extracted, decompose the organic reagent with sulfuric acid, take up in water, and use as sample.

Very Low Lead Content.[33] This treatment of sample was developed for comparison of the lead content of urine of city dwellers with that of country residents, in a study of the effect of "Ethyl" gasoline.

[31] John R. Ross and Colin C. Lucas, *J. Biol. Chem.* 111, 285-97 (1935); F. L. Kozelka and E. F. Kluchesky, *Ind. Eng. Chem., Anal. Ed.* 13, 492-4 (1941).

[32] James E. Kench, *Biochem. J.* 34, 1245-7 (1940).

[33] A. G. Francis, C. O. Harvey and J. L. Buchan, *Analyst* 54, 725-35 (1929).

Prepare nitrosyl sulfuric acid by passing sulfur dioxide into cold concentrated nitric acid until it is saturated. Add 80 ml. of it to 1 liter of urine in small portions, mixing well during the addition. Evaporate to one-third the original volume. Add a few drops of amyl alcohol from time to time to prevent foaming. Add 20 ml. of concentrated nitric acid and evaporate until charring begins. Let cool, add more concentrated nitric acid, and repeat the evaporation until the solution is clear. Evaporate to sulfur trioxide fumes to decompose all nitrosyl sulfuric acid, and continue to heat until separation of calcium salts occurs. Before the mass solidifies, dilute with cold and then hot water to 400 ml. Filter to remove silicic acid. Ash the precipitate with nitric and sulfuric acids. Evaporate to sulfur trioxide fumes. Transfer to a platinum dish and treat with hydrofluoric and sulfuric acids to volatilize silica. This recovers any sorbed lead. Dilute the acid solution and add to the solution of the original sample.

Add 5 ml. of 10 per cent citric acid solution and 4 ml. of 1 per cent copper nitrate solution. Prepare an indicator solution containing 1 gram of methyl orange and 1.4 gram of Xylene cyanol FF in 500 ml. of 50 per cent ethanol. Add 5 drops of this indicator. Add concentrated ammonium hydroxide until the solution passes through a neutral gray color and reaches a green representing a pH of about 4.5.

Saturate with hydrogen sulfide, filter, and discard the filtrate and washings. Destroy the paper by treatment with the minimum possible amount of nitric and sulfuric acids, and evaporate to sulfur trioxide fumes. Add 10 ml. of water, and neutralize with concentrated ammonium hydroxide. Add 15 ml. of water and 1 ml. of concentrated nitric acid.

Electrolyze with a cylindrical gauze anode rotated at 1500-2000 rpm. using 70-80°, 1.5-2.0 volts, and 0.3-0.4 amperes. After 1 hour remove and wash the anode. Dissolve the deposit of lead dioxide in 5 ml. of 1:1 nitric acid and 1 ml. of 95 per cent ethanol. Transfer to a silica beaker and add 1 ml. of concentrated sulfuric acid. Evaporate to fumes and cool. Add 10 ml. of cold water and 5 ml. of 95 per cent ethanol. Mix well and let stand overnight. Filter and wash with 33 per cent ethanol containing 3 per cent of sulfuric acid.

Dissolve the lead sulfate from the paper with 2 ml. of hot 50 per cent ammonium acetate solution and wash the paper well with hot water. Collect the filtrate and dilute if necessary for determination by dithizone.

Although it has been reported that normal urine gives a negative test

for lead,[34] other workers[35] report 0.0005-0.005 mg. per liter in normal individuals, 0.015-0.064 mg. in urine of battery workers, and 0.033-0.394 mg. per liter in cases of chronic lead poisoning.

Feces. Dry the sample in a tared silica or Pyrex dish on a hot plate or steam bath. Heat on an electric plate until volatile matter has been driven off, then ash at 500°. Weigh the cooled dish to determine the amount of ash, then heat with a few ml. of a 3:1 mixture of concentrated hydrochloric and nitric acids. Do not filter to remove silica but rather use the cloudy solution. Excessive washing would be required to remove sorbed lead. Dilute to a known volume and mix well before aliquoting. A proper sample is equivalent to 0.1-0.5 gram of ash. Complete as for urine (page 17), starting at "Transfer 15 ml. of 50 per cent ammonium citrate solution. . . ." Normal persons excrete up to 0.5-1 mg. of lead per day in the feces.[36]

Blood. Weigh a sample into a tared silica or Pyrex dish, and add an equal volume of concentrated nitric acid. Evaporate to dryness and complete as for feces, starting at "Heat on an electric plate. . . ."

Another technic[37] is to heat 5-10 ml. of serum or oxalated blood in a micro-Kjeldahl flask with 1.5 times its volume of concentrated sulfuric acid until all water has been expelled. Add a few crystals of potassium nitrate at 5-10 minute intervals until the sample becomes clear, and heat for 0.5 hour longer. When cool, dissolve the residue in about 10 ml. of water, and again evaporate to fumes to eliminate nitric acid. Take up in water for use as sample for determination by dithizone

A normal lead content of blood is 0.01-0.07 mg. per liter.[38] A concentration as high as 0.32 mg. per liter may not show toxic symptoms.

Tissue.[39] Dry on a sand bath, powder, and heat in a micro-Kjeldahl flask with a minimal volume of sulfuric acid until disintegrated. Com-

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[34] L. Pieti and S. Mangeri, *Medicina lavoro* **27**, No. 2, 33-8 (1936); *Chimie et Industrie* **38**, 453 (1936).

[35] John R. Ross and Colin C. Lucas, *J. Biol. Chem.* **111**, 285-97 (1935); B. Behrens and H. Taeger, *Z. ges. exptl. Med.* **96**, 282 303 (1935).

[36] Harold Traeger and Frida Schmitt, *Z. ges. exptl. Med.* **100**, 717-35 (1937).

[37] H. Kraftström, K. Wulfert and O. Sydnes, *Biochem. Z.* **290**, 382-93 (1937).

[38] R. Massione, *Med. laboro* **31**, 1 18, 25-32, 84 (1940); *Zentr. Gewerbehyg. Unfallverhut.* **28**, 20 (1941); cf. H. Beck and G. Straube, *Klin. Wochschr.* **18**, 242-4 (1939).

[39] For bibliography of treatment of biological samples, see Frederick L. Smith 2nd, Thomas K. Rathmell and Thomas L. Williams, *Am. J. Clin. Path.* **11**, 653-68 (1941).

plete as for the last method for blood starting at "Add a few crystals of potassium nitrate. . . ."

Foods.[40] *Dry ashing.* Contamination in sampling must be rigorously excluded. For mixing or grinding use a porcelain mortar, or metal grinders shown to give no contamination with lead or tin. For stirring to composite samples, use a porcelain or wooden spatula. For sampling from tin containers with soldered seams, open from the bottom to avoid contamination with bits of solder.

This preparation is applicable to carbohydrates, cereals, cacao and dairy products, feeds, meats, fish, plant material, fruits and vegetables and their products, and, in general, organic matter except fats. Special technics must be applied for removal of tin or bismuth if present.

Weigh 5-200 grams of sample, according to the probable lead content, into a porcelain dish or casserole of convenient size. If the product is one difficult to ash, such as meats, add 2-5 ml. of a solution containing 40 grams of aluminum nitrate, $Al(NO_3)_3 \cdot 9H_2O$, and 20 grams of calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$, per 100 ml. If the ash is low and bulk is desired, as in candies and jellies, also make this addition. Mix well and dry.

If the product is one having a tendency to swell, such as gelatin or jam, char by heating over a burner, and control the swelling by playing a flame from a glass jet on the surface. Do not permit the sample to ignite. It is convenient to add milk, candy, etc., in small portions to the dish on a hot plate.

After charring, transfer the dish to a muffle furnace provided with temperature controls. Use asbestos board on the floor to avoid undue heat transfer from the bottom. Raise the temperature slowly. If fat is present, volatilize it by decomposition at about 350°. Finally carry the temperature to 500°, but not above. If the ashing does not proceed satisfactorily within 12 hours, remove the dish and, after cooling, add 2-5 ml. more of the solution of aluminum and calcium nitrates. Dry and replace in the muffle. If now the ashing is not proceeding rapidly within 30 minutes after heating to temperature, again remove, cool, and add 2-3 ml. of concentrated nitric acid. Dry and replace. The final ash should be carbon-free, excessive additions of nitrates and nitric acid

[40] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, pp. 455-464 (1945); cf. G. W. Monier-Williams, Repts. Pub. Health Med. Subjects, Ministry Health, London. No. 88, 51 pp. (1938).

should be avoided. If too much is added, the contents of the dish may deflagrate; in which case restart the determination.

To the cooled, clean ash in the covered casserole, add 15-20 ml. of concentrated hydrochloric acid in small portions. Rinse the cover glass with water, and heat on a steam bath. If a clear solution is not obtained, evaporate to dryness, and take up again in the same way. If still not clear, evaporate again and add 5-10 ml. of 60 per cent perchloric acid. Heat this to fumes to dehydrate silica. Take up in water, using as much as 200 ml. if much potassium is present, and filter through an inorganic crucible into a 500-ml. glass-stoppered flask. Wash the residue on the filter successively with hot concentrated hydrochloric acid, a hot solution containing 20 grams of citric acid and 20 ml. of concentrated hydrochloric acid per 100 ml., and hot 40 per cent ammonium acetate solution.

If an excessive amount of silica is on the filter, transfer it to a platinum dish with water and evaporate to dryness. Add 5 ml. of 48 per cent hydrofluoric acid and evaporate to dryness. Repeat to insure the volatilization of silica. Take up the residue in a few ml. of 1:10 hydrochloric acid and filter into the previous filtrate.

If the ash is low in volume and lead may be baked on the dish, add 5 ml. of 50 per cent sodium hydroxide solution to the dish and heat. Be sure that a film of this covers the area occupied by the sample. Heat on a steam bath for a few minutes but do not take to dryness, since to do so may extract lead from the dish. Take up the solution with water. Neutralize with 1:1 hydrochloric acid and filter into the bulk of the sample. Wash the dish with a few ml. of hot 1:1 hydrochloric acid; then wash the filter.

Transfer the solution of ash to a separatory funnel and add 20 ml. of 50 per cent citric acid solution. Add 1:1 ammonium hydroxide until alkaline to litmus. Cool and let stand for 2-5 minutes.

If the amount of calcium and magnesium phosphates, iron and aluminum hydroxides, etc., in the ash is excessive, so that they cannot be kept in solution with citric acid for extraction with dithizone, separate the lead as the sulfide. For this cool the acid solution of ash and add 20 ml. of 50 per cent citric acid solution. Add 1:1 ammonium hydroxide to a pH of 3.0-3.4 to bromophenol blue. If the amount of lead is small, add 0.5-1 ml. of 1 per cent copper sulfate solution to serve as a collector. Saturate with hydrogen sulfide, filter at once on an inorganic filter, and discard this filtrate. Without washing, dissolve the sulfides in 5 ml. of hot concentrated nitric acid, using the original flask as receiver. Wash with hot water and mix. Boil the filtrate until hydro-

gen sulfide removal is complete, and cool. Add 10 ml. of 50 per cent citric acid solution and make alkaline with 1:1 ammonium hydroxide.

In determination of copper (page 90), a sulfide separation of lead and bismuth is provided which may be dissolved as above and completed by the rest of this method.

To the solution prepared by sulfide separation of lead, or the solution which did not require that operation, add 5 ml. of 10 per cent sodium or potassium cyanide solution. This may be increased if excessive amounts of zinc, copper, cadmium, etc., are present. Add a drop of thymol blue solution to be sure the pH is above 8.5, as shown by a blue-green to blue color. If large amounts of iron are present, as shown by the color, do not raise the pH above 10, as this would oxidize the dithizone. Extract at once with a 20-ml. portion of solution, containing 10 mg. of dithizone per liter of chloroform, by shaking for 10-15 seconds. Let separate and draw off the organic solvent. Repeat until the extract is pure green, the color being unchanged. The lead is now in the dithizone layers.

Add 25 ml. of 1:100 nitric acid to the combined extracts. Shake with the extracts until the organic solvent layer is a pure green, and discard the chloroform layer. The lead is now in the nitric acid solution. Filter this solution through a small wad of wet cotton in the stem of a small funnel, into a 50-ml. calibrated flask. Wash the separatory funnel and small funnel with two 10-ml. portions of the 1:100 acid and make to volume with the same acid.

Note that although dithizone is used for isolation of the lead by this method, and although the recommended AOAC procedure for its determination is by dithizone, the sample is also suitable for application of other methods of determination. If chloroform is objectionable in the aliquot of sample used, heat it to boiling for a moment to drive this off.

Antimony combines with lead as a complex during digestion with sulfuric acid and prevents the lead from forming a dithizonate.[41] If sulfuric acid is absent this does not occur. Thus dry ashing and solution in perchloric and hydrochloric acids is preferred under those conditions.

Lead is also deposited electrolytically from the ash solution and subsequently stripped and determined with dithizone.[42] Interference by silica is unlikely.[43]

[41] F. L. Kozelka and E. F. Kluchesky, *Ind Eng. Chem., Anal. Ed.* 13, 492-4 (1941).

[42] Karl Bambach and Jacob Cholak, *ibid.* 13, 504-5 (1941).

[43] P. A. Clifford, *J. Assoc. Official Agr. Chem* 21, 212-20 (1938)

If ash of baking powder is difficult to dissolve, treat with hot 30 per cent sodium hydroxide solution, followed by an excess of hydrochloric acid.[44]

Meat.[45] Digest 200-500 grams of meat with 200-500 ml. of concentrated nitric acid in a 6-liter Pyrex flask until active foaming has ceased. The resulting uniform solution is not necessarily clear. The digestion on a steam bath should be complete in 30 minutes. Mix well and transfer to a graduated cylinder of suitable capacity. When cool, if floating fat is present, dissolve it in a suitable volume of petroleum ether, such as 25 ml. Pipet an aliquot of the solution corresponding to 5 grams of the original sample into a 100-ml. Kjeldahl flask, avoiding inclusion of the petroleum ether. Add 5 ml. of 60 per cent perchloric acid and digest at a low heat until colorless. Since hot perchloric acid explodes in contact with hot dry organic matter, use more perchloric acid when applying this to samples where more residual organic matter would be present than from meat.

When the solution is colorless, raise the heat and boil until dense white fumes are given off and the residual volume is about 2 ml. Let cool, add 5 ml. of water, and boil for a minute. With canned meat a precipitate of stannic oxide may appear. Cool and add 2 ml. of 20 per cent ammonium citrate solution. Use this solution as sample, preferably by the dithizone method.

Sugar and Sugar Products.[46] Weigh 5-10 grams of sample into a beaker and measure 25 ml. of water into another. Add water from the second beaker to the sample in several portions to dissolve the sample, and transfer to a 500-ml. volumetric flask. When the transfer is complete add 15 ml. of 18:82 hydrochloric acid. Add 10 ml. of 50 per cent citric acid solution and rotate the flask for 5 minutes.

To determine the amount of ammonium hydroxide to be added, mix 30 ml. of water, 15 ml. of the diluted hydrochloric acid, 10 ml. of 50 per cent citric acid solution, and 9 ml. of concentrated ammonium hydroxide. Cool in a water bath to about 20° and add 5 ml. of 20 per cent potassium cyanide solution. Mix and determine the pH by the glass electrode. Add concentrated ammonium hydroxide in 1 ml. portions until the pH is 9.5. The total ammonium hydroxide so added is the amount to be added to the sample.

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[44] P. A. Clifford, *ibid.* 22, 339-41 (1939).

[45] R. M. Melwin, Private Communication.

[46] Thomas D. Gray, *Ind. Eng. Chem., Anal. Ed.* 14, 110-14 (1942).

Add the determined amount of concentrated ammonium hydroxide to adjust to pH 9.5, mix well, and place in a cooling bath. Agitate for 5 minutes and, on removal, at once add 5 ml. of 20 per cent potassium cyanide solution and 125 ml. of a reagent containing 7.5 mg. of dithizone per liter of chloroform. Agitate vigorously for 2 minutes without stoppering. Let stand for 10 minutes for the chloroform layer containing the lead to separate. Pipet 75 ml. of the chloroform layer from the flask by vacuum with a minimum of agitation. Add this to 11 ml. of the diluted hydrochloric acid solution in a separatory funnel and rinse the pipet into the funnel with water. Shake the funnel for 1 minute and let it stand for 10 minutes. Discard the chloroform layer and dilute the aqueous layer containing the lead to a known volume for use of aliquots.

Maple Sirup.[47] Weigh 15 grams of sirup into a container suitable for centrifuging. Add 15 ml. of 18:82 hydrochloric acid. Mix well, add about 25 ml. of water, and mix again. Dissolve 20 grams of potassium cyanide and 10 grams of citric acid in 500 ml. of concentrated ammonium hydroxide and dilute to a liter. Add 15 ml. of this to the solution and mix. Add exactly 15 ml. of a solution of 30 mg. of dithizone per liter in chloroform. Shake 100-200 times and centrifuge. Transfer exactly 11 ml. of the dithizone layer to a separatory funnel containing 11 ml. of the diluted hydrochloric acid. Shake 100-200 times, let separate, and discard the chloroform layer. Use 10 ml. of the aqueous layer by the series of standards method with dithizone reagent.

Milk.[48] Boil 100 ml. of milk and 25 ml. of concentrated nitric acid until the fat-free portion is completely dissolved. Let cool, dilute somewhat, and filter through a wet filter paper. Wash the residual fat on the paper with hot water. Evaporate the filtrate until evolution of nitrogen oxides begins and let cool. Add 20 ml. of concentrated sulfuric acid and heat, with occasional addition of more nitric acid, until all carbon compounds have been oxidized. Finally evaporate to about 2 ml., take up with 10 ml. of water, and again evaporate to 2 ml. Repeat to make sure nitric acid is removed. Transfer to a centrifuge tube, dilute to about 10 ml. and add 10 ml. of ethanol. After standing, centrifuge to separate lead sulfate and calcium sulfate. Decant the upper layer, and wash the residue with a mixture of 3 parts of ethanol to 1 part of 1:12 sulfuric acid. Dissolve the lead sulfate from the well-washed

[47] J. L. Perlman, *ibid* 10, 134 5 (1938).

[48] J. Gangl and E. Liedl, *Mikrochemie, Festschr. von Hans Molisch*, 1936, 147-53.

residue with a measured volume of hot 40 per cent ammoniacal ammonium acetate solution and use this as sample. In application of the dithizone method allow for this amount of ammonium acetate in the procedure.

Beer.[49] Evaporate a 150-ml. sample to dryness and ash. Dissolve the ash in 5 ml. of 1:1 hydrochloric acid and dilute to about 125 ml. with water. Saturate the solution with hydrogen sulfide to precipitate copper and lead. Filter and wash the precipitate with 1:50 hydrochloric acid saturated with hydrogen sulfide. Reserve the solution for determination of iron and tin. Dissolve the mixed sulfides in 2 ml. of hot 1:1 nitric acid and wash the paper thoroughly with hot water. Dilute to a known volume for use of an aliquot. This solution contains the lead and copper.

Organic Samples, Particularly Medicinals.[50] This method of preparation provides not only for lead but also for copper, zinc, and iron. For the usual samples, transfer 2 grams of sample into a 100-ml. Kjeldahl flask, preferably modified as shown in Figure 2. Add 6 ml. of concentrated redistilled nitric acid and 4 ml. of concentrated sulfuric acid. Mix well and warm cautiously until reaction commences. Remove the flame until the initial vigorous reaction is over.

FIG. 2 Special Design of Kjeldahl Recommended

For materials such as methyl violet, which would react violently with a mixture of nitric and sulfuric acid, first treat the sample with 10 ml. of 1:2 nitric acid. After the initial vigorous reaction, decant the acid into a clean beaker. If there is a tarry residue, wash it with several 1-ml. portions of water and add the washings to the acid. Take up any tarry residue in 4 ml. of concentrated sulfuric acid by dropwise addition of concentrated nitric acid, with warming if necessary, until vigorous reaction is over. In the absence of tarry residue simply add 4 ml. of concentrated sulfuric acid to the flask. Return the original acid liquor to the flask.

Whichever technic was used, boil vigorously until the solution begins to darken. At that time add about 3 ml. more of concentrated nitric acid and continue the digestion. Repeat such additions until darkening no longer occurs. Usually 20-25 ml. total of nitric acid will be required.

[49] W. S. Hubbard, *J. Assoc. Official Agr. Chem.* 19, 389-93 (1936).

[50] N. Strafford, P. F. Wyatt and F. G. Kershaw, *Analyst* 70, 232-46 (1945).

When digestion appears complete, add 0.5 ml. of 60 per cent perchloric acid and heat for 15 minutes longer. Add another 0.5 ml. of 60 per cent perchloric acid and heat a few minutes longer. Let cool somewhat, and add 10 ml. of water. Heat to fumes, again cool, and add 5 ml. of water. At this step the sample solution should be colorless. Again heat to fumes, let cool, and add 5 ml. of water.

Prepare a parallel reagent blank by boiling to fumes 4 ml. of concentrated sulfuric acid, the amount of nitric acid added to the sample, and 1 ml. of 60 per cent perchloric acid for about 20 minutes in a 100-ml. Kjeldahl flask. Let cool, add 5 ml. of water, and again take to fumes. Repeat, and finally add 5 ml. of water.

To separate copper and arsenic, transfer the sample solution and blank to flasks. Filter if there is suspended matter present, and in that event use subsequent additions to wash the paper. Rinse out the Kjeldahl flasks with two 1-ml. portions of water. Add 15 ml. of 1:3 hydrochloric acid to each Kjeldahl flask, heat just to boiling, and swirl to flush the sides of the flask. Transfer to the appropriate flask and wash each Kjeldahl flask with two more 1-ml. portions of water. If either mercury or bismuth is present they will also be extracted. If the bismuth is no greater than the copper content, ignore it.

Prepare an iodide solution by dissolving 20 grams of sodium or potassium iodide in 100 ml. of water. Add 0.2 ml. of concentrated ammonium hydroxide solution. Prepare a stock solution of diethylammonium diethyldithiocarbamate. For this, dilute 3 ml. of redistilled diethylamine to 10 ml. with chloroform. Dilute 1 ml of redistilled carbon bisulfide to 10 ml. with chloroform and add to the previous solution. Cool and store in a dark, glass-stoppered bottle. It is stable for about 1 week. For use, dilute 5 ml. of this solution to 100 ml. with chloroform as *carbamate extraction reagent*. This is stable for only a day. Extract the iodide reagent with 10 ml. of the carbamate extraction reagent by shaking for 30 seconds. Discard this extract and wash the iodide reagent with two 5-ml. portions of chloroform.

Add 2 ml. of the iodide reagent to the sample and blank and warm each to about 40° Add 0.5 ml. of clear, 5 per cent sodium metabisulfite solution and transfer each to a 50-ml. separatory funnel. Rinse the flasks with 1-ml portions of water until the volumes in the separatory funnels are 35 ml. Arsenic is now in the trivalent form. The acidity is high enough to prevent extraction of lead. A yellow color not discharged by the metabisulfite is due to bismuth.

Add 5 ml of the carbamate extraction reagent to each and shake for 40 seconds. Loosen the stopper to release pressure and let the layers

separate. Withdraw the solvent layer into a 25-ml. separatory funnel. Wash the aqueous layer with 0.5 ml. of chloroform without mixing and withdraw this into the funnel with the extract. Extract the aqueous layer with 2 ml. of carbamate extraction reagent. Withdraw and wash the aqueous layer as before. Set aside the chloroform extract for later determination of copper and arsenic and proceed with this aqueous layer which now contains lead, zinc, and iron. Evaporate the acid solution until iodine is completely evaporated and acid fumes are evolved. Let cool and add 10 ml. of water.

Prepare a solution of 150 grams of sodium citrate in water. Add 0.5 ml. of concentrated ammonium hydroxide and dilute to 500 ml Extract with 25-ml. portions of 0.02 per cent solution of dithizone in chloroform until the extract remains green. The sodium citrate solution will appear slightly yellow. Add 5 ml. of a 20 per cent solution of citric acid and extract with chloroform until the solution is colorless.

Add 2 ml. of the sodium citrate solution to the sample, 0.2 ml. of 5 per cent sodium metabisulfite solution, then 0.2 ml. of methyl red indicator solution. Almost neutralize by addition of 2:1 ammonium hydroxide,[51] cool well, and finally add 2:1 ammonium hydroxide, dropwise. Add 1:2 hydrochloric acid until definitely acid. Transfer to a separatory funnel and extract with two 5-ml. portions of toluene to remove the indicator. Wash the toluene layers with 1-2 ml. of water without mixing and discard the toluene.

As a dithizone reagent, dissolve approximately 15 mg. in 50 ml. of redistilled toluene. Shake with 50 ml. of 1:50 ammonium hydroxide. Discard the toluene layer and render the aqueous layer acid with 1:2 hydrochloric acid. Extract the dithizone from this with two 50-ml. portions of redistilled toluene. Discard the aqueous layer, combine the toluene extracts, and wash them with two 10-ml. portions of water. This should be prepared freshly for use and is of approximately 0.008 per cent concentration. To the combined aqueous solution and toluene washings add 0.5 ml. of 2:1 ammonium hydroxide, 2 ml. of toluene, and 1 ml. of the dithizone reagent in toluene. Shake for 15 seconds and, unless the reagent changes promptly to a bright pink, add 2:1 ammonium hydroxide dropwise until the color does appear. Continue to add the dithizone reagent in 1-ml. increments until the color changes from bright pink to a purplish shade indicative of the presence of unchanged dithizone. Return the aqueous layer to the flask and reserve it for determination of iron. Wash the toluene layer with 1-2 ml. of water and

[51] The authors call for this to be prepared at 10 N by solution of ammonia gas until the color just changes to yellow.

add these washings to the reserved sample. The toluene layer now contains the lead and zinc as dithizonates.

Add 10 ml. of 0.1 *N* hydrochloric acid to the toluene extract and shake for 30 seconds. Withdraw the acid and wash the toluene with 1-2 ml. of water. Again extract with 10 ml. of 0.1 *N* hydrochloric acid, wash the toluene, and combine the acid extracts and washings. Discard the toluene layer, which will be clear green unless cobalt or nickel is present. To the acid extract add 0.5 ml. of the sodium citrate solution and 0.5 ml. of 2:1 ammonium hydroxide.

Add 0.5 ml. of 10 per cent potassium cyanide solution to the sample and transfer to a separatory funnel. Wash with water and add 2 ml. of toluene and 0.5 ml. of a reagent containing 0.008 per cent of dithizone in toluene. Shake and, if the color is pink, add more 0.5-ml. portions of dithizone in toluene until the shade is duller and the aqueous layer is yellowish with free dithizone. At this stage the lead has been extracted as dithizonate leaving the zinc in the aqueous layer.

Withdraw the aqueous layer. Wash the toluene layer with 1-2 ml. of water and add these washings to the aqueous layer in a separatory funnel. Extract with 2 ml. of toluene. If lead extraction is complete the toluene layer will be a purplish green, the aqueous layer yellow. Add the toluene layer to the lead extraction and reserve the aqueous layer for zinc determination.

Mix 2 ml. of 2:1 ammonium hydroxide and 1 ml. of 10 per cent potassium cyanide solution per 100 ml. of water. Add 10 ml. of this to the lead dithizonate in a separatory funnel. Shake for about 10 seconds. Draw off the aqueous layer and discard it. It should be only pale yellow; if deeply colored repeat the washing. Finally wash with 2 ml. of water. Transfer the toluene extract to a 10-ml. cylinder and dilute to volume with toluene. Filter if necessary to clarify and read the color as an estimation of lead instead of preparing an extract by dithizone as usually provided in the procedure.

Dyes.[52] Boil a 5-gram sample gently with 15 ml. of concentrated nitric acid until evolution of brown fumes has ceased. Add 15 ml. of concentrated sulfuric acid and continue to heat. Add 1-2 ml. of concentrated nitric acid from time to time until organic matter is destroyed and the solution is no more than light yellow. Evaporate to 3-5 ml. and cool. Add 15-20 ml. of water and re-evaporate to fumes. Take up

[52] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, pp. 287-8. Association of Official Agricultural Chemists, Washington, D. C. (1945).

with 100 ml. of water and add 100 ml. of 95 per cent ethanol. After standing overnight filter on a double paper. The precipitate may be invisible. Wash with 100 ml. of 50 per cent ethanol.

Heat the filter to boiling with 20 ml. of 40 per cent ammonium acetate solution and disintegrate with a glass rod. Filter, wash the filter, and use the filtrate as sample for determination as lead sulfide.

Plant Tissue.[53] Weigh 7.5 grams into a beaker and add 12.5 ml. of concentrated nitric acid. Cover with a watch glass and evaporate nearly to dryness on a hot plate. Let cool, add another portion of acid, and repeat the evaporation. Add 25 ml. of 1:1 nitric acid and 25 ml. of 60 per cent perchloric acid. Boil in a covered beaker until nearly dry. Let cool, add 20 ml. of water, and transfer to a platinum dish. Add 5 ml. more of 60 per cent perchloric acid, boil, and add to the platinum dish. Add 2-8 ml. of 48 per cent hydrofluoric acid. Heat on a sand bath until fumes are no longer given off. Cool and transfer to a beaker with hot 1:20 hydrochloric acid, finally using about 100 ml. Crush the residue with a flat-end glass rod and continue to heat. If necessary in order to dissolve the last of the calcium sulfate, add concentrated hydrochloric acid. Finally, dilute to 100 ml. in a volumetric flask. This is solution A for direct determination of molybdenum, manganese, iron, and phosphate.

To separate the lead, and incidentally many other elements present, use the method of extraction by dithizone.[54]

Transfer a 90-ml. aliquot of the ash solution—solution A—to a 500-ml. separatory funnel and add 50 ml. of redistilled water. Prepare 40 per cent ammonium citrate from citric acid and concentrated ammonium hydroxide and adjust to pH 8.5, before extraction with dithizone solution to remove heavy metals. Add sufficient of this to prevent precipitation when the solution is made alkaline, usually 10 ml. Add 1:1 ammonium hydroxide to raise the pH to about 8.5 and a few drops of a 0.1 per cent alcoholic solution of *m*-cresol purple as internal indicator. If the solution turned yellow on addition of citrate it may be necessary to add 15-20 drops of this indicator solution. Add an excess of dithizone reagent containing 0.5 gram per liter of carbon tetrachloride,

[53] R. Q. Parks, S. L. Hood, Charles Hurwitz and G. H. Ellis, *Ind. Eng. Chem., Anal. Ed.* **15**, 527-33 (1943).

[54] E. B. Sandell, *ibid.* **9**, 464-9 (1937); *ibid.* **11**, 364-5 (1939); E. B. Holland and W. S. Ritchie, *J. Assoc. Official Agr. Chem.* **22**, 333-8 (1939); *ibid* **23**, 392-3 (1940); Hale Cowling and E. J. Miller, *Ind. Eng. Chem., Anal. Ed.* **13**, 145-9 (1941); Karl Bambach and Roland E. Burkey, *ibid.* **14**, 904-7 (1942).

usually 20-25 ml., and shake well. The aqueous phase should be orange after shaking. Drain the dithizone solution into a dry separatory funnel and wash the last part through with carbon tetrachloride. Extract the aqueous phase with successive 25-ml. portions of carbon tetrachloride until the nonaqueous layer is green, combining the extracts. This is solution B.

Transfer the aqueous phase to a 200-ml. volumetric flask with 10 ml. of 1:1 hydrochloric acid and reserve this as solution C for later determination of sulfur, calcium, magnesium, potassium, and sodium.

Add 50.0 ml. of 0.02 *N* hydrochloric acid to solution B in a 500-ml. separatory funnel. Shake vigorously for 2 minutes and draw off the carbon-tetrachloride phase into a beaker. Rinse the funnel with carbon tetrachloride and add this to the beaker. This is solution D and contains the copper and cobalt. Set aside for working up for those elements.

Draw off the aqueous phase into a dry container, dilute to a known volume as solution E, and use aliquots for determination of zinc, cadmium, and lead, preferably by the dithizone method.

Medicinal Iron Preparations. Mix a 2-gram sample, or more if the copper is under 20 ppm., with 5 ml. of water and 10 ml. of concentrated sulfuric acid. Heat gently and add 5 ml. of 30 per cent hydrogen peroxide to oxidize organic matter. Bring to a boil for 5 minutes and let cool slightly. Add 5 ml. more of 30 per cent hydrogen peroxide and again bring to a boil for 5 minutes. If necessary, add more hydrogen peroxide until oxidation is complete. About 15 ml. is normally required. Cool and add 5 ml. of water and 10 ml. of concentrated hydrochloric acid. Boil until clear. Cool and add a solution of 10 grams of citric acid in 50 ml. of water and 30 ml. of concentrated ammonium hydroxide. Again cool and add 1:2 ammonium hydroxide until neutral to litmus. Add 10 ml. of this ammonium hydroxide in excess and transfer to a separatory funnel. Extract with 3 successive 10-ml. portions of chloroform containing 0.1 gram of dithizone per 100 ml. If an emulsion forms, disperse it with ethanol. Combine the extracts and wash 3 times with 50 ml. of water. The color of the wash water is due to excess reagent. Transfer to a small flask and evaporate the chloroform. Cool and add 0.5 ml. of concentrated sulfuric acid. Add a few drops of concentrated nitric acid and heat to destroy organic matter. If necessary, add a few more drops of nitric acid. To complete the removal of nitric acid add a few ml. of water to the solution after it cools and heat until white fumes are given off.

The cooled residue contains the copper nnd lead present in the original sample. Add 10 ml. of water to dissolve. Add 1 gram of citric acid and 4 grams of ammonium acetate. When solution is complete, add 1:1 ammonium hydroxide until alkaline to litmns and dilute to 100 ml.

Antimony-Bispyrocatechol-3,5-Sodium Disulfonate. *Stibophen.*[55] Treat 1 gram with 10 ml. of concentrated nitric acid and 5 ml. of concentrated sulfuric acid. After the initial reaction, heat to white fumes and a clear, nearly colorless liquid. Let cool and carefully dilute with 15 ml. of water. Add 1 gram of tartaric acid and cool. Neutralize with 20 per cent sodium bydroxide solution and use as n sample by the sulfide method.

Gasoline.[56] Although tetraethyl lead in gasoline is usually susceptible of gravimetric or volumetric determination, when the concentration is sufficiently low colorimetric methods are applicahle.

Pipet 100 ml. of gasoline at 15.5° into a flask, adding 0.1 ml. for each degree the temperature is above that value, or subtracting 0.1 ml. for each degree below. If more than 75 per cent of the gasoline distills below 100° it is necessary to cut 1:1 with straight-run kerosene. Add 50 ml. of concentrated hydrochloric acid and connect with a reflux condenser. Heat rapidly to boiling and reflux gently for 30 minutes. Hydrochloric acid fumes are lost through the condenser. Let cool and separate the gasoline and aqueous layers. Reflux the gasoline with two 50-ml. portions of water, adding the aqueous layers to the acid.

Evaporate the acid solution to a dry residue of lead cbloride. Add 30 ml. of concentrated nitric acid and evaporate to dryness to oxidize any organic materials. Repeat if the dry lead salt is not white. Dissolve the lead nitrate in 10 ml. of 1:10 nitric acid, dilute to a known volume, and use an aliquot. To express as tetraethyl lead, 1 ml. = 1.0570 gram of lead. The original reference sbows special equipment for running large numbers of samples.

Alternatively,[57] beat 20 ml. of sample for a few minntes with 2 ml. of concentrated nitric acid. Pour off the acid layer and reserve. Repeat the extraction 3 times with 1-ml. portions of concentrated nitric acid. To the combined extracts add 2 ml. of concentrated snlfuric acid dropwise and boil gently for a few minutes. Cool and add 5 ml. of saturated

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[55] *British Pharmacopoeia.* 3rd Addendum (1941).

[56] George Calingaert and C. M. Gambrill, *Ind. Eng. Chem., Anal. Ed.* **11**, 324-5 (1939).

[57] Georges Schuster, *Ann. chim. anal.* **25**, 55-6 (1943).

ammonium tartrate solution, 1 ml. of saturated ammonium acetate solution, and 15 ml. of water. Determine lead by the sulfide method.

Separation of Copper, Zinc, Bismuth, Lead, and Tin.[58] The sample to which this separation is applied may be the clear, colorless one from wet digestion which has been evaporated to fumes of sulfur trioxide and taken up with water. It may be a solution in hydrochloric acid of ash obtained by incineration. As outlined, it is based on about 20 grams of original sample.

To the solution of sample add 2 grams of citric acid and 0.01 gram of ferrous sulfate. When dissolved, add concentrated ammonium hydroxide until neutralized to about pH 8.0. The use of an internal indicator is permissible. Pass hydrogen sulfide through the solution for 20 minutes, let stand for a few minutes, and filter. The ferrous sulfide serves as a collector. Wash the precipitate with saturated aqueous hydrogen sulfide solution to which concentrated ammonium hydroxide has been added until it is distinctly ammoniacal. The use of yellow ammonium sulfide would result in low values for zinc. All the ammonium citrate must be removed. The filtrate with washings is the sample for determination of tin. The hydrogen sulfide need not be removed for determination as sulfide or with dithiol (toluene-3,4-dithiol).

Dissolve the black sulfide precipitate by gently warming in 20 ml. of 1:10 nitric acid. When the sulfides have completely dissolved replace the same paper in the funnel and filter the solution through it. Wash well with hot water to give a total volume of about 50 ml. Add 1 gram of ammonium sulfate and heat nearly to boiling. Add 15 ml. of 1:6 ammonium hydroxide and continue to heat until the ferric hydroxide has coagulated. *If the solution is boiled, ammonia will be lost and copper carried down with the ferric hydroxide.*[59] Filter and wash the precipitate on the filter with 1:50 ammonium hydroxide.

Dissolve the precipitate by warming the paper with 20 ml. of 1:10 nitric acid, transfer the paper to the original funnel, and filter this solution. Wash the filter well with hot water and make the filtrate up to 50 ml. in a volumetric flask in order to use aliquots for determination of bismuth and lead. Dilute the filtrate to 100 ml. in a volumetric flask and use aliquots for determination of copper and zinc.

Separation of Bismuth and Lead. Since the two elements are quite closely related it is evident that problems of separation will arise.

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[58] J. Hubert Hamence, *Analyst* 62, 18-23 (1937).

[59] J. Hubert Hamence, *Trans. Faraday Soc.* 30, 299-303 (1934).

The most efficient separation is by dithizone whose lead salt is extracted at pH 7.5, the bismuth salt at pH 2.5. Transfer an aliquot of sample containing up to 0.5 mg. of bismuth. Dilute to about 20 ml. with water and add 1:1 ammonium hydroxide until the solution is alkaline to methyl red. Although the solution is red, any precipitate may be pink. Add phenol red to it to be sure the solution is still acid. Heat on a water bath, cool, filter into a separatory funnel, and wash with water. Add 3 ml. of 50 per cent ammonium citrate solution, 1 ml. of 10 per cent potassium cyanide solution, 0.5 ml. of 20 per cent hydroxylamine hydrochloride solution, and 1:1 ammonium hydroxide solution until alkaline to phenol red. At this pH, which will be 7.5 or higher, extract the lead and most of the bismuth with 3 successive 5-ml. portions of chloroform containing 30 mg. of dithizone per liter. Discard the aqueous layer and wash the combined extracts with 50 ml. of water. Wash the water with 5 ml. of chloroform and add this to the extracts. Shake the combined extracts with 20 ml. of 1:100 nitric acid and discard the chloroform layer.

Add 1:1 ammonium hydroxide to this acid layer until the pH is about 2, which is orange with metacresol purple. Extract bismuth with successive 5-ml. portions of the dithizone reagent until the last remains a pure green. Discard these extracts. Wash the aqueous layer with 5 ml. of chloroform and discard the washings. The remaining aqueous solution is the solution for analysis.

Lead may also be separated as the dioxide.[60] Concentrate the sample solution to about 40 ml. and, if not already present, add 4-6 grams of ammonium nitrate. Add 1:1 ammonium hydroxide until the blue color of the copper-ammonia complex is just detectable. Clear the blue color by addition of a single drop of 1:1 nitric acid. Transfer to a platinum dish as cathode and use a rotating gauze anode. Warm to about 33° and electrolyze at about 0.5 ampere and 2.3 volts for 20 minutes. With the current still on, withdraw the dish from the anode and wash it with water.

For methods where lead is determined as the dioxide, dissolve directly from the anode in the reagent. For others dissolve in a few ml. of sulfurous acid and take up with 1:15 nitric acid.

Standard

Recrystallize lead nitrate, $Pb(NO_3)_2$, repeatedly from water and dry at 110° to constant weight. Dissolve 1.599 gram in 1:100 nitric acid and

[60] Herbert Müller, *Z. anal. Chem.* **113**, 161-82 (1938).

dilute to 1 liter with the same acid. This contains 1 mg. of lead per ml. Dilute 10 ml. to 100 ml. with 1:100 nitric acid for 0.1 mg. per ml., or 10 ml. to 1 liter for 0.01 mg. per ml. Do not store these dilute solutions more than a week.

Lead by Dithizone

The importance of the application of dithizone to lead [61] to give a rose-red color [62] is illustrated by its use with over 1,000,000 samples in a year.[63] Good correlation with spectrographic and polarographic results is obtained.[64] The comparable di-β-naphthylthiocarbazone gives a purple color. The reaction is of 1 mol of lead with 2 mols of dithizone.[65]

Ferric ion tends to oxidize the reagent. Freedom from such oxidizing agents and the products of oxidation is essential.[66] The presence of aluminum ion interferes and titanium ion prevents development of the color.[67] By extraction of the lead at pH 9-10 in the presence of cyanide, it is separated from reasonable amounts of all interfering reactive substances except bismuth and thallous ions. Stannous tin must be oxidized.[68] Iron must be repressed by complex formation such

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[61] Hellmut Fischer, *Wiss. Veröffent. Siemens-Konzern* **4**, 158 (1925); *Z. angew. Chem.* **42**, 1025-7 (1929); *Mikrochemie* **8**, 319 (1930); Noel L. Allport and G. H. Skrimshire, *Analyst* **57**, 440-9 (1932); *Pharm. J.* **129**, 248 (1932); Hellmut Fischer, *Angew. Chem.* **46**, 442-6 (1933); Hellmut Fischer and Grete Leopoldi, *Wiss. Veröffent. Siemens-Konzern* **12**, 44-52 (1933); *Angew. Chem.* **47**, 90-2 (1934); A. J. Hijman, *Acta Brevia Neerland. Physiol., Pharmacol., Microbiol.* **4**, 148-0 (1934); G. Roche Lynch, R. H. Slater and T. G. Osler, *Analyst* **59**, 787-806 (1934); Sidney L. Tompsett and Alan B. Anderson, *Biochem. J.* **29**, 1851-64 (1935); H. J. Wichmann, *J. Assoc. Official Agr. Chem.* **18**, 182-9 (1935); E. S. Wilkins, Jr., C. E. Willoughby, E. O. Kraemer and F. L. Smith, 2nd, *Ind. Eng. Chem., Anal. Ed.* **7**, 33 6 (1935); C. E. Willoughby, E. S. Wilkins Jr., and E. O. Kraemer, *ibid.* **7**, 285-6 (1935); O. B. Winter, Helen M. Robinson Frances B. Lamb and E. J. Miller, *ibid.* **7**, 265-71 (1935); Paul A. Clifford, *J. Assoc. Official Agr. Chem.* **20**, 191-4 (1937); Hellmut Fischer, *Angew. Chem.* **50**, 919 32 (1937); F. L. Kozelka and E. F. Kluchesky, *Ind. Eng. Chem., Anal. Ed.* **13**, 484-7, 492-4 (1941); Arnold Lassieur, *Chim. Anal.* **29**, 88-9 (1947).

[62] Donald M. Hubbard, *Ind. Eng. Chem., Anal. Ed.* **12**, 768-71 (1940).

[63] H. J. Wichmann, *ibid.* **11**, 66-72 (1939).

[64] H. Taeger and F. Schmitt, *Arbeitschutz* 1937, 154-5.

[65] Paul A. Clifford, *J. Assoc. Official Agr. Chem.* **26**, 26-53 (1943); cf. Herman A. Liebhafsky and Earl H. Winslow, *J. Am. Chem. Soc.* **59**, 1966 71 (1937).

[66] Sidney L. Tompsett, *Analyst* **61**, 591-7 (1936).

[67] Joseph Schultz and Melvin A. Goldberg, *Ind. Eng. Chem., Anal. Ed.* **15**, 155 8 (1943).

[68] A. V. A. Munton, H. H. Wittenberg and G. K. Crowell, *J. Am. Water Works Assoc.* **37**, 207 8 (1945).

as with cyanide.[69] Citric acid is generally used for forming complexes. Either tartaric or acetic acid offers advantages with regard to ease of extraction of the lead at high pH levels.[70]

The oxidative effect of traces of ferric ion on the reagent can be overcome with hydroxylamine hydrochloride.[71] This is inadequate when appreciable amounts of iron are present, but extraction with dithizone, then treatment with dilute nitric acid, followed again by the reagent, will suffice.

When potassium cyanide does not remove interference by iron, it can be removed by extraction as the cupferron complex. If so, the excess of cupferron in the lead solution is equally objectionable. The solution from which the iron was extracted must be evaporated to fumes to destroy the cupferron.[72] Precipitation of iron from the ash of biological samples as ferric hydroxide in the presence of ammonium acetate and urea [73] may occlude lead.[74]

Sulfur dioxide will simultaneously preserve the dithizone from oxidation, reduce ferric iron, and oxidize tin. Then the lead can be recovered in a single extraction, provided bismuth is absent.[75] Bismuth can be extracted by dithizone from lead at pH 2.0 [76] or 3.0-3.5.[77] When large amounts of calcium are present it is preferable to isolate the lead as sulfide. This avoids the use of large amounts of ammonium citrate to keep it in solution. While citrate buffer holds phosphate in solution, such interference can also be prevented by addition of excess magnesium sulfate in ammoniacal solution. The precipitate need not be filtered.[78] Manganese in strongly alkaline solution catalyzes oxidation of dithizone by air. Much zinc interferes unless precipitated by ferrocyanide in solution faintly alkaline with ammonium hydroxide.

Sometimes the presence of substantial amounts of tin must be eliminated. It can be overcome by dry ashing with nitric acid to convert it

[69] H. Cheftel and M. L. Pigneaud, *Ann. fals.* 29, 76-92 (1936).

[70] L. P. Biefeld and T. M. Patrick, *Ind. Eng. Chem., Anal. Ed.* 14, 275-8 (1942).

[71] B. Behrens and H. Taeger, *Z. ges. exptl. Med.* 96, 282-303 (1935); F. L. Kozelka and E. F. Kluchesky, *Ind. Eng. Chem., Anal. Ed.* 13, 492-4 (1941).

[72] M. L. Panouse-Pigneaud and H. Cheftel, *Ann. fals.* 32, 296-301 (1939).

[73] René Fabre and Francoise Lem, *Ann. méd. legale criminol. police sci.* 16, 433-6 (1936).

[74] Committee Report, *Analyst* 60, 541 (1935).

[75] F. L. Kozelka and E. F. Kluchesky, *Ind. Eng. Chem., Anal. Ed.* 13, 492-4 (1941).

[76] M. K. Horwitt and George R. Cowgill, *J. Biol. Chem.* 119, 553-64 (1937).

[77] V. A. Gant, *Ind. Med.* 7, 608-22, 679-99 (1938).

[78] Hans Westerhoff, *Bodenkunde u. Pflanzenernähr* 7, 370-84 (1938).

to metastannic acid.[79] If to be volatilized, solution of the ash in perchloric acid facilitates removal as stannic bromide on treatment with bromine and hydrobromic acid.[80] The extraction of lead dithizonate from thallium dithizonate at pH 6.0-6.4 is feasible.[81]

The monocolor method is only difficultly reproducible and its use for lead is inadvisable.[82] In extraction, between 25 and 50 per cent excess of dithizone is necessary as a minimum to have all of the lead recovered. Although the duplication method is sometimes applied,[83] it is far from the most convenient.

Lead dithizonate in carbon tetrachloride at 0.001 mg. of lead per ml. has a maximum absorption at about 520 mμ and absorption by dithizone in carbon tetrachloride is at a minimum. The spread in reading the color by the mixed color method is doubled by use of a 610 mμ filter in place of 510 mμ[84] as predicted by curves.[85] Reading at 660 mμ is very satisfactory.[86] The filter combination of Wrattan 45 and 58, which transmits 10 per cent at 510 mμ but only 0.1 per cent at 478 mμ and 550 mμ is also satisfactory. The method has been applied with the step photometer.[87] The limiting factor in this method is probably the reagent blank. In obtaining the blank all steps must be followed, including the period of ashing by whatever means is used. By avoiding filtrations through cotton or paper and minimizing reagents as much as possible the blank on the photometric reading has been reduced to as little as 0.001 mg.[88]

In any of the versions of this method, if the curve was prepared with the same batch of reagents, it provides correction for contamination except in preparation of the sample.

Procedure. *Elimination of Interference by Bismuth.* This is provided for in some preparations of sample (page 8). When necessary, adjust the pH of the sample extract to about pH 2.0 with 1:20 ammonium hydroxide, or 1:20 hydrochloric acid, using metacresol purple as indi-

[79] E. P. Laug, *J. Assoc. Official Agr. Chem.* 21, 481-7 (1938).
[80] H. J. Wichmann and P. A. Clifford, *ibid.* 18, 315-27 (1935).
[81] Paul A. Clifford, *ibid.* 26, 26-53 (1943).
[82] L. P. Biefeld and T. M. Patrick, *Ind. Eng. Chem., Anal. Ed.* 14, 275-8 (1942).
[83] M. K. Horwitt and G. R. Cowgill, *J. Biol. Chem.* 119, 553-64 (1937).
[84] Charles L. Guettel, *Ind. Eng. Chem., Anal. Ed.* 11, 639-40 (1939).
[85] Paul A. Clifford and H. J. Wichman, *J. Assoc. Off. Agr. Chem.* 19, 130-56 (1936).
[86] F. L. Kozelka and E. F. Kluchesky, *Ind. Eng. Chem. Anal. Ed.* 13, 484-7 (1941).
[87] F. Morton, *Analyst* 61, 465-71 (1936).
[88] Karl Bambach, *Ind. Eng. Chem., Anal. Ed.* 11, 400-3 (1939).

cator. Add 10 ml. of a solution of 250 mg. of dithizone per liter of chloroform. Shake for a minute and allow to separate. If the lower layer is orange-red to red, extract with another 10 ml. of the reagent. Continue to extract with 5-ml. portions, shaking for 3-5 minutes, until the extracts remain pure green. Dilute the aqueous layer to a known volume for use of an aliquot. When using as sample, adjust the pH of the aqueous layer, from which bismuth and stannous ions have now been removed.

Series of Standards. Transfer 25 ml. of 1:100 nitric acid to each of 10 separatory funnels. Add varying amounts of standard lead nitrate solution and sufficient of the acid to make each to 50 ml. The usual range for such a sample is 0.001-0.005 mg. but higher ranges can be used. Place one or more suitable aliquots of sample in similar funnels. If not acid, neutralize to litmus and add sufficient 1:10 nitric acid to make the solution 1:100 with nitric acid. Dilute the standard or standards to 50 ml. with 1:100 nitric acid. Dilute 100 ml. of 10 per cent sodium or potassium cyanide solution with sufficient redistilled ammonium hydroxide to add 19.1 grams of ammonia, and then to 500 ml. with water. Add 10 ml. of this to each sample and standard. This gives a pH of about 9.7. Refer to Table 1 for the amount and concentration of dithizone solution to use. Add this and shake for 1 minute. As a check on the sufficiency of the reagent added, the color in the chloroform layer should show some green along with the red of copper dithizonate. Withdraw the chloroform layers into suitable Nessler tubes and observe through or lengthwise according to color intensity. Avoid exposure to strong sunlight. Beyond a limit the dithizone solutions also appear red by transmitted light. Standards, if protected, keep at least a day. Storage in a refrigerator is most satisfactory.

TABLE 1. RELATION OF AMOUNT OF LEAD TO CONCENTRATION AND VOLUME OF DITHIZONE SOLUTION TO BE USED

Lead (mg.)	*Dithizone (mg./liter) in chloroform*	*Volume Dithizone Solution*	*Cell Length (inches)*
0-0.005	4	5	2
0.005-0.010	4	10	2
0.010-0.020	8	10	1
0.020-0.050	8	25	1
0.050-0.100	10	30	0.5
0.100-0.200	20	30	0.5

If a sample is outside the range of the standards it may be extracted with more reagent and transferred to nitric acid solution again starting on page 23 at "Add 25 ml. of 1:100 nitric acid to the combined extracts." Then use a smaller aliquot. Unless the contamination is in preparation of the sample, the blank is automatically taken care of.

Photometric.[89] Add to the sample, or aliquot, a solution of 2.5 mg. of dithizone per 100 ml. of chloroform in 1-ml. increments, shaking after each addition, until the lower layer shows a noticeable purple to green color indicative of excess dithizone. Add chloroform to make the total volume of the solvent layer to 10 ml. Shake well, let separate, and withdraw the chloroform layer. Discard the sample solution.

Prepare an extraction solution containing 10 ml. of 5 per cent potassium cyanide solution and 5 ml. of concentrated ammonium hydroxide per 500 ml. Shake the chloroform layer with 20 ml. of this. Discard the extraction solution so used and repeat the washing. This will remove excess reagent. Read the transmittance of the chloroform layer at 520 mμ and compare with a curve after correcting for a blank. A parallel method of determination[90] with standardized conditions is to read the excess dithizone at 610 mμ.

Monocolor Method.[91] In this method excess dithizone is extracted before reading. In general it is less accurate. Shake the chloroform extracts with successive 5-ml. portions of 0.5 per cent potassium cyanide solution until the chloroform extract shows no green. Two washings are usually sufficient. Aside from direct reading, this permits conversion to the equivalent amount of dithizone for reading. For this purpose shake the carbon tetrachloride solution with 5 ml. of approximately 0.2 N nitric acid until the color in the chloroform is a pure green. Separate the chloroform layer and read at around 610 mμ.

Extraction at pH 11.5.[92] To the sample in aqueous solution and free from interfering ions add 4 drops of 0.1 per cent thymol blue indicator solution. Add 10 ml. of 5 per cent ammonium citrate solution, then 1:1 ammonium hydroxide until the color becomes pale blue, indicating a pH of 9.5-10.0. Add 10 ml. of 2 per cent potassium cyanide solution

[89] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 328-30, American Society for Testing Materials, Philadelphia, Pa.

[90] Charles L. Guettel, *Ind. Eng. Chem., Anal. Ed.* 11, 639-40 (1939).

[91] Hellmut Fischer and Grete Leopoldi, *Z. anal. Chem.* 119, 161-88 (1940).

[92] L. J. Snyder, *Anal. Chem.* 19, 684-7 (1947).

and extract with successive 20-ml. portions of a solution of 60 mg. of dithizone per liter of chloroform. Add the successive extracts to 25 ml. of 1:100 nitric acid. When the green color of a portion remains unchanged, extract with 5 ml. more of the dithizone solution. Each 20 ml. portion was equivalent to approximately 0.45 mg. of lead. The extractions will therefore have given an approximate measure of the lead present.

Shake the extracts with the nitric acid and discard the solvent layer. Evaporate the droplets of chloroform from the surface and read the color of this aqueous acid solution at 510 mμ. If necessary, dilute with 1:100 nitric acid to reduce the lead concentration. It is convenient to vary the length of the cell used for reading and let the intensity of color vary over a wide range.

Double Conversion.[93] Extract the sample or aliquot with successive 5-ml. portions of dithizone solution, containing 12 mg. per liter of chloroform, until the extracts are unchanged in color. Usually 3 extractions suffice unless the lead exceeds 0.05 mg. Extract the combined chloroform solution with 20 ml. of 1:100 nitric acid and discard the chloroform layer.

To the 20-ml. acid layer, add 4 ml. of a solution containing 15 ml. of concentrated ammonium hydroxide and 2 grams of potassium cyanide per 100 ml. Add 2 drops of 20 per cent hydroxylamine hydrochloride solution and 10 ml. of a solution containing 12 mg. of dithizone per liter of chloroform. Shake vigorously for 0.5 minute and allow to separate. Filter the chloroform layer directly through a dry paper into a test tube or cuvette. Read the transmittance with a 530-mμ filter and compare with a standard curve.

Lead as the Sulfide

A limited amount of lead in solution on the addition of hydrogen sulfide will not precipitate but will give a brown color of the colloidal sulfide.[94] Electrolytes affect the size of the lead sulfide particles in colloidal suspension and therefore the nature as well as the intensity of color developed. The British Pharmacopoeia reports that at 7-12 grams

[93] Joseph Schultz and Melvin A. Goldberg, *Ind. Eng. Chem., Anal. Ed.* 15, 155-8 (1943).

[94] J. C. Thresh, *Analyst* 49, 124-8 (1924); Constantin Pyriki, *Z. anal. Chem.* 64, 325-30 (1924); Merle Randall and Marian N. Sarquis, *Ind. Eng. Chem., Anal. Ed.* 7, 2 3 (1935); Georg Gad, *Gas-u. Wasserfach* 79, 105-0 (1936); G. W. Monier-Williams, *Repts. Pub. Health Med. Subjects, Ministry Health, London* No. 88, 51 pp. (1938).

of electrolyte per liter the effect is substantially uniform. A colloidal material, such as gelatin, will not only stabilize the suspension but also influence the color produced. Many other metals which form dark sulfides must be absent or tied up in a stable complex. Copper, for example, can be displaced by zinc, or tied up as the cyanide complex. Interference by traces of iron is also prevented by cyanide. The preparation of sample is often as complex as for the dithizone method, but this method is applicable to lead contents in the higher range of 0.005-0.25 mg. per 100 ml.

The applications are diverse such as to water of varying degrees of contamination,[95] urine, cream of tartar, solder,[96] preserved meat,[97] beer,[98] edible oil[99] and other foods,[100] and tissue.[101]

Procedure. *Series of Standards.* To each of a series of standards and to the sample in 50-ml. Nessler tubes, add 1:1 ammonium hydroxide to neutrality and a 3-ml. excess, then 1 ml. of 10 per cent potassium cyanide solution. If sulfide is present in the potassium cyanide, add 2 ml. of 3 per cent hydrogen peroxide to each 100 ml. of 10 per cent solution just before diluting to final volume. Let this stand 24 hours before use. The peroxide oxidizes sulfide to sulfate. Mix well and dilute to nearly 50 ml. Then add 1 ml. of 1 per cent colorless sodium sulfide solution, mix, dilute to volume, and compare.

Duplication. Considering the method of preparation of the sample, prepare a blank containing the same reagents. To sample and standard in 50-ml. Nessler tubes add 1:1 ammonium hydroxide to approximate neutrality, and 3 ml. in excess. Then add 1 ml. of 10 per cent potassium cyanide solution and dilute each to about 45 ml. Mix and add 1 ml. of 1 per cent colorless sodium sulfide solution. Dilute the sample to 50 ml., add an appropriate standard lead solution to the standard, and finally adjust the standard to 50 ml. Repeat preparation of the standard, adding the lead standard before the sulfide, as a check on the duplication carried out.

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[95] J. F. Reith and J. deBens, *Chem. Weekblad* 32, 205-10 (1935); Hayo Bruns and Karl Heinz Tänzler, *Gesundh.-Ing.* 59, 485-7 (1936); J. W. Hawley and W. Wilson, *Analyst* 62, 166-72 (1937).

[96] L. I. Bockarova, *Voprosy Pitaniya* 5, No. 5, 147-50 (1936).

[97] N. V. Shirokov and D. S. Mindlina, *Z. Untersuch. Lebensm.* 70, 245-51 (1935).

[98] Percy G. Jackson, *J. Soc. Chem. Ind.* 56, 211-13T (1937).

[99] Vizern and Guillot, *Ann. chim. anal. chim. appl.* 19, 258-60 (1937).

[100] E. N. Sergeeva, *Voprosy Pitaniya* 6, No. 1, 103-12 (1937).

[101] F. Gallego y Gomez, *Anales soc. españ. fís. quím.* 33, 937-41 (1935).

Lead as the Chromate by s-Diphenylcarbazide

The high degree of insolubility of lead chromate or a double salt permits its precipitation, filtration, and estimation of lead from the combined chromate radical.[102] The lead chromato may be separated, redissolved in acid, and the chromate color estimated.[103] Another method is estimation indirectly of the excess chromate in the filtrate with s-diphenylcarbazide.[104] That most satisfactory is direct estimation of the chromate combined with the lead as the double chromate, by s-diphenylcarbazide. Precipitation conditions must be carefully controlled as the double lead-potassium chromate only precipitates at pH 6.6-7.4. Its use is desirable as giving greater sensitivity of the method. The composition is variable down to 5.4, and below that normal chromate is formed. Failure to control precipitation conditions is probably a major source of difficulty in unfavorable comments.[105] The method possesses the advantage of ease of manipulation. The sample may contain mercury, copper, silver, bismuth, nickel, cobalt, arsenic, calcium, barium, strontium, magnesium, manganese, zinc, cadmium, tin, aluminum and iron, and separation of lead will still be satisfactory.[106]

Procedure. Add phenol red indicator to a sample solution containing about 0.01 mg. of lead, and then add 1:1 ammonium hydroxide until the color of the indicator is altered to pink. Filter into a 15-ml. centrifuge tube, washing the original container and paper with four 1-ml. portions of 1:140 ammonium hydroxide. Carefully add 1:8 acetic acid, dropwise, to change the indicator to orange-yellow. To a similar tube add 1 ml. of lead standard containing 0.01 mg. of lead per ml. and approximately the same reagents as are present in the sample.

To sample and standard add 1 ml. of 40 per cent ammonium acetate solution, washing down the sides of the tube. Add 1 ml. of 30 per cent

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[102] B. Jones, *Analyst* 55, 318-20 (1930); Edward W. Krans and J. B. Ficklen, *J. Ind. Hyg.* 13, 140-3 (1931); T. V. Letonoff and John G. Reinhold, *Ind. Eng. Chem., Anal. Ed.* 12, 280-4 (1940); *ibid.* 13, 631 (1941).

[103] L. S. Van der Vlugt, *Chem. Weekblad* 25, 194 (1928); P. W. Danckwortt and E. Jürgens, *Arch. Pharm.* 266, 374-82 (1928).

[104] A. Cazeneuve, *Analyst* 25, 331 (1900); A. Moulin, *Bull. soc. chim.* 31, 295 (1904); B. Breteau and P. Fleury, *J. pharm. chim.* 10, 265 (1914); B. S. Evans, *Analyst* 46, 285 (1921).

[105] Laurence T. Fairhall, *J. Ind. Hyg.* 15, 289 (1933); Robert A. Kehoe, Frederick Thamann and Jacob Cholak, *J. Ind. Hyg. Toxicol.* 18, 42-68 (1936); Jacob Cholak, Donald M. Hubbard, Robert R. McNary and Robert V. Story, *Ind. Eng. Chem., Anal. Ed.* 9, 488-90 (1937).

[106] Z. Karaoglanov and M. Michov, *Z. anal. Chem.* 103, 113-19 (1935).

potassium chromate solution. Mix by stirring but avoid scratching the walls of the tube. Cover to stand overnight. The precipitate which forms is the double salt, lead potassium chromate.

Remove the stirring rod and wash any precipitate into the tube with about 3 ml. of 0.4 per cent ammonium acetate solution. Centrifuge for 10 minutes to separate the chromate. Decant the supernatant liquid and invert the tube to drain for 5 minutes. Dry the lip of the tube. Add 10 ml. of 0.4 per cent ammonium acetate solution, washing all the inner walls of the tube, and suspend the precipitate by stirring. Wash the stirring rod off with about 3 ml. of 0.4 per cent ammonium acetate solution and centrifuge the tube. Decant the supernatant liquid, drain the tube for 5 minutes, and wipe the mouth of the tube.

Add 3 ml. of 1:3 hydrochloric acid, washing the walls of the tube, and stir until the precipitate is dissolved. Add 10 ml. of 0.02 per cent solution of s-diphenylcarbazide, stopper, and mix by inversion. Let stand for 10 minutes for color development. Compare with a series of standards, or read through a 540 mμ filter and compare with a prepared curve. Each mol of chromate ion is equivalent to 0.5 mol of lead.

Lead by Tetramethyldiaminodiphenylmethane

Small amounts of lead converted to the form of lead dioxide, as by electrodeposition, may be used for oxidation of tetramethyldiaminodiphenylmethane to a blue diphenylmethane dye.[107] The lead content is estimated from the intensity of this color. The method will detect 0.05 mg. of lead. The presence of traces of iron and aluminum is not objectionable and it is probable that a great many other elements do not interfere.

Procedure. Place a sample of appropriate size on a boiling water bath and add saturated bromine water until a permanent yellow color is obtained. Sodium hypochlorite or sodium persulfate may also be used for oxidation. Hydrogen peroxide will not oxidize the lead. Heat for 2 hours. Lead will be precipitated as lead dioxide. Filter while hot on a Gooch crucible, first washing the crucible by filtering a few ml. of bromine-water to avoid the presence of reducing agents. Wash thor-

[107] M. A. Trillat, *Compt. rend.* 136, 1205-7 (1903); M. Klostermann, *Naturwissenschaften* 14, 1116 8 (1926); A. Necke, P. Schmidt and M. Klostermann, *Deut. med. Wochschr* 52, 1855 6 (1926), A. Seiser, A. Necke and H. Muller, *Arch. Hyg.* 99, 158-64 (1928); A. D Petrov, *J. Russ. Phys. Chem Soc.* 60, 311-6 (1928); M. V. Neustrueva, *Trav. inst. état radium* (U.S.S.R.) 4, 304 12 (1938); Herbert Müller, *Z. anal. Chem* 113, 161 82 (1938).

oughly to remove traces of residual bromine which will also oxidize the reagent. An alternative sample is the platinum electrode on which lead has been deposited electrolytically.

Prepare a 1.0 per cent solution of tetramethyldiaminodiphenylmethane in glacial acetic acid. This is stable for 24 hours. Filter 25 ml. of this solution through the crucible or apply to the electrode. The glacial acetic acid used as solvent for the reagent dissolves the lead dioxide and the latter oxidizes the reagent to give a blue color. In dissolving lead dioxide in the reagent, the latter should be added in small portions and each completely washed out before adding the next portion. The last treatments should remain colorless, indicating that solution of lead dioxide is complete.

Compare with a series of standards prepared by the action of known amounts of lead dioxide on the reagent, or with a single standard by dilution. Alternatively read the transmittance at around 600 mμ and compare with a calibration curve.

Lead Dioxide as the Molybdate

Lead dioxide may be converted to lead molybdate which gives an orange color suitable for colorimetric estimation, with stannous chloride and a thiocyanate.[108] As developed, it is for application to lead dioxide electrolytically deposited. The order of addition and mixing of the reagents is important. Manganese peroxide present with the lead peroxide does not interfere. If any permanganate color develops, remove it with hydrogen peroxide.

Procedure. Moisten the anode having a deposit of lead dioxide with 1:1 hydrochloric acid, drop by drop. Wash thoroughly with hot 1:100 hydrochloric acid. The precipitate goes into solution. Dilute the solution to 30-40 ml. with water and neutralize with 1:4 ammonium hydroxide. Acidify with 0.5 ml. of glacial acetic acid. Heat the solution to boiling and add 10 ml. of 0.5 per cent ammonium molybdate solution. Boil for 4-5 minutes to coagulate the precipitate. Filter on a small pad of moist filter paper laid over the opening of the stem of a filtering funnel.

Wash 5-6 times with 1:100 acetic acid. Dissolve the precipitate in boiling hot 10 per cent sulfuric acid. This will require 7-8 additions of 5-ml. portions. Cool the solution and transfer to a volumetric flask.

Add 10 ml. of a 5 per cent potassium thiocyanate solution and mix. Follow this with 5 ml. of 10 per cent stannous chloride in 1:4 hydro-

[108] S. Feinberg, *Z. anal. Chem.* 96, 415-8 (1934).

chloric acid and mix again. Dilute to 100 ml. with 10 per cent sulfuric acid. A more or less intense orange-yellow color develops which reaches the maximum in a few minutes and is stable for several hours. Compare, by balancing, with a standard which is very close in color to the sample and was developed at the same time.

Lead Nephelometrically by Sodium Bisulfite

In the presence of copper, nickel, iron, aluminum, silver, calcium and magnesium, lead can be estimated by the turbidity produced by reaction with sodium bisulfite.[109] Barium interferes by precipitation as sulfite or sulfate, as does stannous ion. The method has been successfully applied with a photoelectric nephelometer. The method will detect 1 ppm. The lead may be in solution in ammonium acetate.

Procedure. To 50 ml. of sample add 50 ml. of a freshly prepared, neutral 2 per cent solution of sodium bisulfite. Let stand for a few minutes for turbidity to develop. Either compare with a series of standards prepared at the same time or read in absolute terms against prerecorded results of standards which have stood for the same length of time.

Red Lead as Lead Dioxide

This is a separate method applied to paint pigments containing more or less red lead.[110] The reaction is first to dissolve the red lead in anhydrous acetic acid,

$$Pb_3O_4 + 8CH_3COOH \rightarrow Pb(CH_3COO)_4 + 2Pb(CH_3COO)_2 + 4H_2O.$$

The lead tetraacetate is then hydrolyzed on dilution to give colloidal lead dioxide suitable for colorimetric estimation. Anhydrous conditions up to the time for dilution are essential. The system conforms to Beer's law. There are no interfering substances known.

Procedure. *Red Lead* Dry the sample *in vacuo* at 125°. Transfer a 0.5-gram sample to a dry 125-ml. glass-stoppered flask. Add exactly 20 ml. of fresh, glacial acetic acid from a buret. Stopper and swirl a

[109] V. N. Ivanov, *Chem.-Ztg.* 38, 450 (1914); W. B. S. Bishop and T. Cooksey, *Med. J. Australia* 2, 660-2 (1929); T. Cooksey and S. G. Walton, *Analyst* 54, 97-9 (1929); Rollo K. Newman, *Med. J. Australia* 1, 781-5 (1930); E. A. Leibman, *Lab. Prakt. (U.S.S.R.)* 16, No. 10-11, 23-4 (1941).

[110] Melvin H. Swann, *Anal. Chem.* 19, 191 (1947).

few minutes. To complete solution, loosen the stopper and warm at 60° until reaction is complete, swirling every few minutes. Cloudiness or a dark coloration indicate that the conditions were not anhydrous.

Accurately measure 0.25-3.0 ml. into a beaker. Add exactly 5 ml. of absolute ethanol per 0.25 ml. of aliquot and swirl for 2-3 seconds. Color here indicates water in the alcohol. Add 225 ml. of cool water with immediate and rapid stirring. Dilute to 250 ml., read with a green filter, and compare with a calibration curve.

Mixed Pigments. Remove the pigment as usual except use anhydrous ether for extraction. Dry and sieve through 100-mesh or finer. Dry for several hours at 125° *in vacuo*. Transfer a sample containing at least 0.2 gram of red lead to a predried 50-ml. centrifuge tube with a constricted neck. Add exactly 20 ml. of glacial acetic acid. Close with a rubber stopper and shake for a few minutes. Loosen the stopper to relieve pressure and warm at 60°, shaking occasionally. After 15 minutes, centrifuge until the upper layer is clear.

Transfer 1 ml. to a beaker. Quickly add 20 ml. of absolute ethanol, swirl for a second, and immediately add 200 ml. of water with mixing. Dilute to 250 ml. and read with a green filter. Fading starts in 10-15 minutes. If the color is too faint, use a larger aliquot of the centrifugate, with not less than 20 ml. of ethanol per ml. of acetic acid. Delay in adding water after the ethanol is added will cause quicker fading.

Miscellaneous

Nephelometric determination as the chromate [111] will detect 0.006 mg. of lead.[112] Zinc must be absent. The usual parallel technic with sample and standards is as follows: Evaporate to dryness with a drop of concentrated nitric acid. Take up with water, filter, and transfer the filtrate to a comparison tube. Add 1 drop of 6 per cent acetic acid and 1 drop of 10 per cent potassium chromate solution. Dilute to 100 ml., mix and compare. A suitable range of standards is 0.03-0.60 mg. of lead per 100 ml.

Oxidation of aniline by lead dioxide to a purple color has also been applied colorimetrically.[113]

[111] B. S. Evans, *Analyst* 53, 267 75 (1928).

[112] L. S. van der Vlugt, *Chem. Weekblad* 25, 194-6 (1928); P. W. Danckwortt and E. Jürgens, *Arch. Pharm.* 266, 374-82 (1928).

[113] Walter V. Morgan, *J. Ind. Eng. Chem.* 11, 1055 (1919).

CHAPTER 2

THALLIUM

The reactions of thallium are similar to those of lead, to which it is closely related. It is commonly found with lead, zinc, iron, tellurium, and the alkalies, and often occurs in substantial concentration in the dust of the flues from pyrites burners. The only important use is as the poisonous ingredient in rodenticides.

Many polyvalent ions in their higher stage of valence in the presence of iodide ion are reduced to the lower valence with liberation of iodine. This is the case with iron and copper as well as with thallium. Fortunately, few ions other than iron interfere under the test conditions for the major method of estimation of thallium—liberation of iodine by reduction of thallic to thallous ion. A wide range of sensitivity is possible. A complex thioglycollate on complete precipitation and separation may be dissolved for reduction of phosphotungstomolybdic acid to the familiar molybdenum blue. Thallous ion with excess phosphomolybdic acid forms a stable yellow hydrosol.

Sample

Samples can in general be prepared by technics described for lead. For methods other than that as the sulfide it is rarely necessary to remove other heavy metals. When essential the separation can be made by one of the methods which follow.

Separation with Dithizone.[1] Use 50 ml. of neutral sample solution, free from organic matter. Add a few drops of 10 per cent hydroxylamine hydrochloride solution to insure reduction to the thallous condition. Add 0.5 gram of potassium cyanide and 0 5 gram of ammonium citrate, if not already present in preparation of the solution. The pH at this stage must be 9-12. Extract successively with four 15-ml. portions of 0.1 per cent solution of dithizone in chloroform (page 3). Lead, stannous, and bismuth ions must be absent or they will be coextracted. Nickel, cobalt, mercury, and zinc retard the extraction and no more than 0.1 gram of them should be present. Combine the extracts and wash with

[1] L. A. Haddock, *Analyst* 60, 394-9 (1935).

an equal volume of water. All of the thallium is in the chloroform layer. Discard the aqueous solution and washings. Evaporate the chloroform solution to dryness in a micro-Kjeldahl flask. Add 1 ml. of concentrated sulfuric acid and heat to destroy the dithizone. During the period of heating add 30 per cent hydrogen peroxide drop by drop to the hot acid mixture. When colorless, let cool and take up with 20 ml. of water for use as a sample free from heavy metals. The method by liberation of iodine is preferred.

Separation by Ether.[2] Use 25 ml. of sample solution free from organic matter. Mix with an equal volume of concentrated hydrochloric acid, allowing for any acid already present. Add sufficient chlorine water to bleach any slight color and give a substantial excess. Usually about 5 ml. will be required. This maintains the thallium in the thallic condition. Thallous salts are insoluble in ether. Ferric iron is extracted, but later addition of phosphate prevents interference from that source.

Extract thoroughly with 50 ml. of ether, followed by two 25-ml. portions. Combine the ether extracts and, if the sample solution contained a heavy metal in addition to thallium, extract with 25 ml. of 1:1 hydrochloric acid. Discard this acid extract. Transfer to and evaporate in a narrow-necked flask. Add 15 ml. of water, a few drops of concentrated hydrochloric acid, and 2 ml. of concentrated sulfuric acid to the residue in the flask. Insert a short-stem funnel in the neck of the flask to prevent mechanical loss and evaporate on a hot plate to fumes of sulfur trioxide. Add concentrated nitric acid or 30 per cent hydrogen peroxide, a drop at a time, to the solution in sulfuric acid, while still on the hot plate. The operation is complete when a colorless or light yellow solution is obtained. A few drops of oxidizing agent are usually sufficient. If necessary to maintain fluidity, add a known additional volume of sulfuric acid.

Cool and add 30 ml. of 15 per cent ammonium chloride solution to destroy nitrites.[3] If additional concentrated sulfuric acid has been added, add 15 ml. more of 15 per cent ammonium chloride for each additional ml. of acid. Evaporate to dryness over a free flame, rotating to prevent spattering. Dissolve in about 20 ml. of water as the sample, or dilute to a known volume and use an aliquot.

[2] A. A. Noyes, W. C. Bray and E. B. Spear, *J. Am. Chem. Soc.* 30, 515-17 (1908); E. L. Baldeschweiler, *Ind. Eng. Chem., Anal. Ed.* 4, 101 (1932); Paul A. Shaw, *ibid.* 5, 93-5 (1933).

[3] G. H. Nelson, Max Levine and J. H. Buchanan, *Ind. Eng. Chem., Anal. Ed.* 4, 56 (1932).

Use of an equivalent ether extraction of thallic bromide from hydrobromic acid separates it from all metals except gold.[4]

STANDARD

Dissolve 0.1304 gram of thallous nitrate in water and dilute to 1 liter. Each ml. contains 0.1 mg. of thallium. Dilute 10 ml. of this solution to 100 ml. Each ml. of this diluted solution contains 0.01 mg. of thallium. For more dilute standards, again dilute 10 ml. to 100 ml. to give 0.001 mg. of thallium per ml. Renew the standards at frequent intervals.

THALLIUM BY LIBERATION OF IODINE

A thallium salt in the presence of free chlorine or bromine is maintained in the thallic condition. So treated, after removal of excess halogen, addition of potassium iodide to the acid solution results in quantitative liberation of two atoms of iodine per atom of thallium much like the reaction of ferric chloride and iodide. In this case a thallous compound is formed. Copper, lead, arsenic, mercury, tungsten and molybdenum do not interfere. Traces of iron can be tolerated. Thallium chromate cannot be analyzed by this method.

The iodine so liberated may be estimated in any conventional way, as discussed at length in Chapter 53. Extraction of the iodine with an organic solvent and its colorimetric determination has been shown to be 97 per cent efficient.[5]

Such solutions of iodine in organic solvents follow Beer's law. By this method an accuracy of 1-5 per cent was obtained on 0.5-mg. amounts in 20 grams of meat. Similar accuracy was obtained on thallium-coated wheat. Accuracy to 1 per cent was obtained on thallium salts. An alternative is to develop the blue color with starch in the aqueous solution.[6] These methods of colorimetric estimation can be carried out much more rapidly than volumetric or gravimetric determination.

Procedure. *Extraction.* Transfer the neutral sample solution, or an aliquot, and a suitable standard, to 150-200 ml. narrow-neck flasks. Add 1 gram of ammonium chloride, if not already present, and dilute to about 20 ml. Add 10 ml. of concentrated hydrochloric acid and 10 grams of disodium phosphate to 90 ml. of saturated bromine-water. Add 25 ml.

[4] Isaburo Wada and Raizo Ishii, *Bull. Inst. Phys. Chem. Research* (Tokyo) 13, 264-74 (1934).

[5] Paul A. Shaw, *Ind. Eng. Chem., Anal. Ed.* 5, 93-5 (1933).

[6] L. A. Haddock, *Analyst* 60, 394-9 (1935).

of this reagent to sample and standard. Boil vigorously for 3 minutes while rotating over a free flame. At this stage substantially all the excess bromine should have been removed. Prolonged boiling will give low results. Therefore, in routine use, the minimum time for visual removal of bromine should be determined. A residual trace can be tolerated. The thallic chloride or bromide present is more stable in acid solution. This also increases the efficiency of iodine extraction. The phosphate eliminates the effect of traces of ferric ion.[7]

Cool and transfer to a 125-ml. separatory funnel. Add 0.25 ml. of a 25 per cent solution of phenol in glacial acetic acid and let stand for 5 minutes. This reacts with any residual bromine. Dilute to about 60 ml. Add 5 ml. of 0.2 per cent potassium iodide solution and 20 ml. of carbon disulfide to the separatory funnel. Stopper and shake for 15-30 seconds. Permit the layers to separate and withdraw the carbon disulfide solution of iodine. The volume of carbon disulfide used should be modified with different samples according to the probable thallium content so as to give a proper concentration of iodine after extraction. When such modification is made the volumes of bromine and potassium iodide solutions should also be modified. This need only be approximate.

Compare the sample and standard similarly treated, using all possible precautions to minimize evaporation of the solvent. Standards can be kept for 24-48 hours when properly stoppered. Alternatively read the transmittance of the sample at 600 mμ and compare with a calibration curve.

By Starch. As starch reagent, make a paste of 1 gram of soluble starch in 5 ml. of water, and add with stirring to 45 ml. of boiling water slowly enough so that the boiling does not stop. Add 50 ml. of glycerine in the same way and boil gently for 5 minutes. Cool and adjust the volume to 100 ml.

Prepare the sample as for extraction through "The phosphate eliminates the effect of traces of ferric ion." Dilute the cooled sample and standard to 35 ml. in 50-ml. stoppered cylinders. Add 5 drops of a 25 per cent solution of phenol in glacial acetic acid and mix well. The phenol reacts with any trace of free bromine which may still remain. After 3 minutes add 5 ml. of a fresh 0.2 per cent solution of potassium iodide and 1 ml. of the starch reagent. Mix well and let stand for 5 minutes at 18°. Compare the sample and standard. If the transmittance is read photoelectrically use a filter centering around 600 mμ. The blue

[7] R. Fridli, *Deut. Z. ges. gericht. Med.* **15**, 478-88 (1930).

Lovibond glasses are a suitable artificial standard when calibrated against standard samples.

Thallium as the Sulfide

When thallium has been separated from other ions giving insoluble sulfides, it can be determined in a way parallel to that for lead, using neutral or ammoniacal solution.[8] The sulfide is brown. Cyanide does not interfere with its formation.

Procedure. Transfer 10 ml. of sample solution into a 100-ml. Nessler tube and 10 ml. of an appropriate standard thallium salt solution into a duplicate tube. To each add sufficient 10 per cent potassium hydroxide solution to neutralize to phenolphthalein, and 50 ml. of water. Add 3 ml. of 5 per cent sodium sulfide solution to each and dilute to 100 ml. with water. Mix well and compare by dilution or balancing. The standard and sample should not vary by more than 20 per cent.

Thallium by Phosphotungstomolybdic Acid

Thallium can be precipitated by thionalide, thioglycollic β-amino-naphthalide, in cyanide solution. A solution of the precipitate is used to reduce phosphotungstomolybdic acid on warming in the presence of formamide, to give an equivalent of the usual molybdenum blue.[9] Accuracy to 20 per cent is readily obtained, and a higher percentage if the concentration of the sample is close to that of the standard.

Procedure. Use a suitable volume of thallium solution free from organic matter, usually 5 or 10 ml., in a centrifuge tube In a similar tube take a suitable standard solution. Add 8 per cent sodium hydroxide solution until each is just alkaline to phenolphthalein, and 0.5 ml. in excess. Add 0.5 ml. of fresh 10 per cent potassium cyanide solution and mix well. Add a fresh 5 per cent solution of thionalide in acetone, dropwise until precipitation is complete in each, and a couple of drops in excess. The usual amount required is about 0.3 ml. To obtain a coarsely crystalline precipitate, heat the loosely-stoppered tube in a water bath at 90° for 5-10 minutes. Let cool and centrifuge. Decant the clear supernatant layers and add 3 ml. of acetone to each. Mix well and decant the washings to waste. Repeat this washing twice more.

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[8] Conrad Stich, *Pharm. Ztg.* 74, 27-9 (1929).

[9] Richard Berg and W Roebling, *Angew. Chem.* 48, 430-2, 597-601 (1935); Richard Berg, E. S Fahrenkamp and W. Roebling, *Mikrochemie, Festschr. von Hans Molish* 1936, 42-51.

Prepare the reagent by boiling 18 ml. of water, 5 ml. of sirupy phosphoric acid, 5 grams of sodium tungstate, and 1 gram of phosphomolybdic acid under an all-glass reflux for 2 hours. The solution should be completely clear.

Add 2 ml. of 95 per cent ethanol and 2 drops of 1:20 sulfuric acid to the centrifuge tubes containing the precipitates. Warm to dissolve, if necessary. The centrifuge tubes, if uniform in size and graduated, may serve as comparison tubes, by dilution, or the solutions may be transferred. If transferred, reserve 1 ml. of alcohol to rinse the tubes. Add 2 ml. of water and mix. Add 3 drops of the reagent and 2 ml. of formamide. Mix and place in a water bath at 40° for 15 minutes. Compare the sample with the standard, after dilution with water to a volume which gives a suitable intensity of color.

Thallium as the Phosphomolybdate

A thallous solution acidified with nitric acid reacts with phosphomolybdic acid to form a yellow thallous phosphomolybdate.[10] In the presence of excess phosphomolybdic acid this remains as a yellow hydrosol which cannot be removed by filtration or centrifuging.

A significant color is given by 0.004 mg. per ml. Lead, bismuth, mercuric, and cadmium ions in large excess do not interfere under the conditions of the test. Mercurous ion gives a faint precipitate in dilute nitric acid which disappears on further addition of acid. Potassium and ammonium salts should be absent because of formation of relatively insoluble salts with phosphomolybdic acid. The yellow colloid dissolves in hydrochloric acid, sodium hydroxide, or sodium carbonate solutions on warming. Accuracy to 1-5 per cent is obtained.

Procedure. To 5 ml. of neutral sample containing 0.002 to 0.01 mg. of thallium per ml. and to a similar volume of a suitable standard add 3 drops of 1:1 nitric acid and 3 drops of a 5 per cent solution of phosphomolybdic acid. Mix and, after 5 minutes, dilute to 10 ml. and compare.

Miscellaneous

Thallous dithizonate is extractable by chloroform above pH 10.[11] No method of quantitative estimation by dithizone extraction has been developed but such a method in parallel to that for lead appears logical. General details of dithizone methods follow page 1.

[10] F. Pavelka and Hermine Morth, *Mikrochemie* 5, 30-3 (1932).
[11] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66-72 (1939).

CHAPTER 3

SILVER

Silver, aside from minor contaminating amounts in minerals, occurs in association with lead. Some pharmaceutical preparations contain it. Special solders contain silver. It is an ingredient of photographic materials and is even attaining an increased importance in water sterilization for swimming pools and drinking water. About 0.03-0.06 mg. are required per liter.[1] In addition to all of these uses, it is employed chemically in many atomic weight determinations. Silver even occurs biologically as a result of treatments. Its accidental occurrence is relatively infrequent.

The classical method of determination is nephelometrically as the chloride, but the versatile dithizone method and several others are applicable. Pyrex glass should be used for all determinations of dilute solutions made at room temperature, and for all procedures where heat is applied, fused silica should be used. Soft glass may cause as much as a 20 per cent loss of silver due to sorption.[2]

Samples

Minerals.[3] To 1.0 gram of finely divided sample add 5 ml. of contrated nitric acid and bring to the boiling point. Cool, decant the clear upper layer, and put aside. Repeat the nitric acid treatment 3 additional times. Finally, add 5 ml of concentrated nitric acid to the residual mineral and evaporate to dryness on a steam bath. Cool and take up in 10 ml. of 1:20 nitric acid solution. Combine with the nitric acid extracts, add 2 volumes of water, and filter.

To the filtrate add saturated bromine water until the solution is yellow, then add 10 ml. of concentrated hydrobromic acid. Evaporate slowly until the silver bromide coagulates. Filter the precipitate on a sintered glass crucible and wash with 10 ml. of distilled water. Place

[1] Irl C. Schoonover, *J. Research Natl. Bur. Standards* 15, 377-84 (1935); William E. Caldwell and Kenneth N. McLeod, *Ind. Eng. Chem., Anal. Ed.* 9, 530-2 (1937).

[2] F. Haber, *Z. angew. Chem.* 40, 303-14 (1927); H. Freundlich and K. Soellner, *Biochem. Z.* 203, 3 (1928), Franz Quittner, *Ann. Physik* [IV] 85, 745-69 (1928).

[3] C. F. Miller, *Chemist Analyst* 25, 8-10 (1936).

the crucible in a beaker and cover with 10 ml. of concentrated ammonium hydroxide. Allow to stand for 4 hours and filter into a 100-ml. volumetric flask. Wash with 30 ml. of 1:100 ammonium hydroxide, then with distilled water. Dilute to volume for the use of aliquots. This sample cannot be acidified for use. It is suitable for reduction with sodium hyposulfite.

Lead Minerals Containing Silver.[4] Treat 0.2-0.3 gram of the powdered mineral with 5 ml. of concentrated nitric acid free from chlorides. Heat gently to dissolve, then continue heating on a steam bath to evaporate to dryness. Add 2 ml. of 10 per cent potassium sulfate solution, 3 ml. of water, and 5 ml. of ethanol. Chill, filter the lead sulfate precipitate, and wash with five 2-ml. portions of ethanol. Evaporate the filtrate to dryness and take up in 10 ml. of water. Filter into a 25-ml. volumetric flask and wash the precipitate with water. Dilute the combined filtrate and washings to volume.

Acids and Lemonades.[5] Evaporate a 100-ml. sample to dryness. Cool somewhat, add 0.5 gram of sodium nitrate, and fuse to destroy organic material. Cool, dissolve the melt in water, and dilute to a suitable volume. Determine preferably by the *p*-dimethylaminobenzalrhodanine method.

Solutions. *Cobalt, Nickel, Cadmium or Copper Present.* To a known volume of a solution containing silver add an excess of bromine water and concentrated hydrobromic acid. Evaporate until the silver bromide coagulates. Filter the precipitate, which may also contain lead or thallium, on an inorganic filter and wash with water. Place the crucible in a beaker and add 10 ml. of concentrated ammonium hydroxide. Cover with a watch glass and let stand for 4 hours. Wash the crucible with 1:100 ammonium hydroxide and dilute the resulting solution and washings to a silver concentration of about 10 mg. per liter. This must not be acidified for use.

Cobalt, Nickel, Cadmium and Copper Absent. Transfer 200 ml. of sample solution to a 250-ml. volumetric flask. Add 5 ml. of bromine water, mix, and set aside for 3 minutes. Add 2 ml. of 10 per cent diammonium phosphate solution and 0.5 ml. of saturated sodium hypo-

[4] Const. G. Makris and Raoul Menaché, *Ann. chim. anal. chim. appl.* 22, 117-20 (1940).

[5] O. Noetzel, *Z. Untersuch. Lebensm.* 78, 315-21 (1939).

sulfite solution. Mix, add 2 ml. of concentrated ammonium hydroxide, and mix again. Dilute to 250 ml. and, after 10 minutes, filter through a dry filter. Reject the first 25 ml. of filtrate. This treatment removes magnesium, calcium, iron, and aluminum, and lends itself to determination of silver by the hyposulfite method.

Water. Use without further preparation or, if the silver content is so low as to make it necessary, acidify with a few drops of 1:1 nitric acid and concentrate in a quartz dish.

Lymph.[6] Dry a sample of lymph on the water bath. Ash carefully, and fuse with 0.2-0.4 gram of sodium nitrate until the residue is white. Heat with two 5-ml. portions of 1:1 nitric acid for 5-10 minutes to dissolve everything but silver chloride. Transfer to a 100-ml. volumetric flask. Wash the residue with two 5-ml. portions of 1:10 ammonium hydroxide, add to the filtrate, and dilute to volume for use of aliquots.

Standards

Dry pure silver nitrate crystals in a desiccator. Dissolve 0.1575 gram in water and dilute to 1 liter. Each ml. corresponds to 0.1 mg. of silver. For 0.01 mg. per ml. dilute 10 ml. to 100 ml. and use within 24 hours.

Silver Nephelometrically as the Chloride

The optical properties of silver chloride suspensions, which are used in the nephelometric estimation of small amounts of silver, are influenced by the conditions under which they are prepared. Variations in these conditions, such as the rate of addition of the chloride, amount of shaking, and the presence of electrolytes,[7] can make reproduction of results poor.[8] Instrumental errors also cause deviations.[9] However, with due precautions this method has even been used in the determination of atomic weight.[10] A photronic nephelometer improves the reproductibility of results.[11]

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[6] P. W. Danckwortt, *Arch. Pharm.* 252, 29-76 (1914); Tibor V. Heidlberg, *Biochem. Z.* 192, 238-40 (1928).

[7] A. F. Scott and John L. Moilliet, *J. Am. Chem. Soc.* 54, 205-9 (1932); I. M. Kolthoff and Henry Yutzy, *ibid.* 55, 1915-22 (1933).

[8] P. V. Wells, *Chem. Rev.* 3, 331-82 (1927); I. M. Kolthoff and Henry Yutzy, *J. Am. Chem. Soc.* 55, 1915-22 (1933).

[9] Hans Kleinmann, *Biochem. Z.* 99, 115-49 (1919).

[10] T. W. Richards and R. C. Wells, *Am. Chem. J.* 31, 235 (1904); A. B. Lamb, P. W. Carleton and W. B. Meldrum, *J. Am. Chem. Soc.* 42, 253 (1920).

[11] Charles H. Greene, *J. Am. Chem. Soc.* 56, 1269-72 (1934).

There is a tendency for shaking and cooling to leave an excess of chloride ion in solution, which may amount to several tenths of a mg. per liter.[12] Some sources of the error are sorption of ions by silver chloride during peptization and coagulation, differences in the coagulating action of the two precipitating reagents, and peptizing and coagulating effects of other compounds present.

Adding the precipitant dropwise or pouring the sample into the precipitant cannot be duplicated with sufficient accuracy. The more rapidly the mixing takes place the less the turbidity. However, reproducibility is better with slower methods of mixing.[13] Special equipment permits reproducibility with very rapid mixing.[14]

Suspensions formed by the addition of excess silver or excess chloride ions to a saturated silver chloride solution maintain a constant turbidity for the first 90 minutes. Those suspensions prepared by mixing the reagent and solution uniformly attain a constant turbidity almost immediately, whereas those prepared by adding the reagent dropwise require about 10 minutes before reaching equilibrium. The amount of silver present in the chloride solution before precipitation has little effect on the turbidities produced when the chloride is precipitated in a solution where the chloride concentration is equivalent to that of a saturated silver chloride solution at 0°.

Procedure. To 20 ml. of acid or neutral solution containing silver ion, add 10 ml. of 1:160 nitric acid. Then add 10 ml. of 0.005 *N* hydrochloric acid. Mix well, heat on a water bath at 40° for 30 minutes, cool rapidly to room temperature, and compare nephelometrically with a standard prepared at the same time in the same way.

Silver by Dithizone

If silver dithizonate is formed in neutral or alkaline solution, it is in a red-violet enol form which is not quantitatively extractable with organic solvents. When formed in acid solution the resulting yellow color is the keto form. This is not converted to the enol by making its solution or dispersion strongly alkaline.

Therefore a carbon tetrachloride solution of dithizone[15] added to an acid solution of silver ions extracts a yellow color proportional to the

[12] Clyde R. Johnson, *J. Phys. Chem.* **35**, 540-2, 830-5, 2237-44, 2581-4 (1931).

[13] Arthur F. Scott and John L. Moilliet, *J. Am. Chem. Soc.* **54**, 205-9 (1932).

[14] Arthur F. Scott and Frank H. Hurley, *ibid.* **56**, 333-5 (1934).

[15] For a more detailed discussion of this reagent and precautions necessary in its use refer to page 3.

silver content.[16] Unlike most other metallic ions, extraction is complete at all pH levels above about 1.5.[17] The stability to alkali leads logically to extraction of excess dithizone with ammonium hydroxide solution when the monocolor method is preferred to the mixed-color determination. In the latter the color is green to yellow, determined photometrically through a yellow filter. The color develops with dithizone in aqueous pyrophosphate solution.[18] The pyrophosphate acts to keep interfering metals, except mercury, in solution as colorless complexes.

By working in acid solution when the color is developed, interference by metals such as lead, zinc, and bismuth, which react in basic solution, is avoided. Mercury gives the same color as silver under substantially the same conditions. The interfering ion can be left behind when the silver dithizonate is extracted. The sample must be free of gold, palladium, and bivalent platinum. If copper is present a special procedure is necessary in which a violet solution of copper dithizonate is used as reagent. By use of this special reagent, copper used as a collector in isolation of silver need not be separated for application of this method. Halides and thiocyanates must be absent. By suitable choice of sample, amounts of silver from 0.001 mg. up are determined.

Procedure. *Mixed-color Method.* Transfer an aliquot of sample, containing 0.005-0.025 mg. of silver, to a separatory funnel. Adjust the acidity to about 0.5 *N*. A convenient method is to dilute the sample to about 25 ml. and approximately neutralize. Then add 0.7 ml. of 1:1 sulfuric acid.

Add 10 ml. of a 0.001 per cent solution of dithizone in carbon tetrachloride and shake well. The reagent layer will show a residual green color unless too much silver was present in the sample. Separate the layer, and read the transmittance through a yellow filter. Compare with a calibration curve similarly obtained. The series-of-standards and duplication methods are also applicable.

With Cupric Dithizonate. As reagent, shake 25 ml. of 0.001 per cent dithizone in carbon tetrachloride with dilute copper sulfate solution, slightly acidified with sulfuric acid, until no further color change occurs. Separate the carbon tetrachloride layer and wash with 25 ml. of water

[16] Hellmut Fischer, Grete Leopoldi and Horst von Uslar, *Z. anal. Chem.* 101, 1-23 (1935).

[17] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66-72 (1939).

[18] R. I. Alekseev, *Zavodskaya Lab.* 7, 415-17 (1938).

to which a drop of 1:1 sulfuric acid has been added. This violet reagent is not altered in color by copper in the sample solution.

Prepare the sample as for the mixed-color method. Add 10 ml. of the reagent and shake for 1 minute. Separate the yellowish-orange to violet tinged reagent layer, read the transmittance with a yellow filter, and compare with a calibration curve. Alternatively the series of standards or duplication methods are applicable.

Monocolor Method. Prepare the sample as for the mixed-color method. Add 5 ml. of a reagent containing 0.005 per cent of dithizone in carbon tetrachloride. Shake well, let separate, and withdraw the reagent layer. Extract with 2 ml. portions of the reagent until the last portion remains unchanged in color. The silver is now all in the combined reagent extracts. Wash the sample solution with 1 ml. of carbon tetrachloride and add this to the extracts. Discard the aqueous sample.

Wash the silver dithizonate solution in carbon tetrachloride with two 10-ml. portions of 1:1000 sulfuric acid. Wash out excess reagent by shaking with 1:1000 ammonium hydroxide in 10-ml. portions until the wash solution is colorless. Dilute the silver dithizonate solution to a known volume with carbon tetrachloride, and filter if necessary to remove water droplets. Read the transmittance in a photometer at 460 mμ, or balance against a standard similarly prepared.

Silver by 2-Thio-5-keto-4-carbethoxy-1,1,3-dihydropyrimidine

In dilute acid solution, 2-thio-5-keto-4-carbethoxy-1,1,3-dihydropyrimidine [19] forms a deep purple, insoluble compound with silver,[20] which is the basis for a fairly specific method for the determination of small quantities of the metal.[21] The reagent is pink in basic solution, orange in neutral solution, and yellow in acid solution. The intensity of the yellow color is little affected by the pH. The reaction takes place most readily in approximately neutral solutions having a low concentration of electrolytes. Under these conditions, cadmium, ammonium, copper, iron, lead, zinc, manganese, and tin also react. At lower pH values, less interference is encountered and greater stability obtained. Nitric acid proves an especially sensitive and selective medium. Mercury interferes and must be absent. The limiting concentrations of other ions per ml. at

[19] Eastman Kodak Company, Rochester, New York.

[20] S. E. Sheppard and H. R. Brigham, *J. Am. Chem. Soc.* 58, 1046-9 (1936).

[21] John H. Yoe and Lyle G. Overholser, *Ind. Eng. Chem., Anal Ed.* 14, 148-9 (1942).

low pH are: cobaltous 0.5 mg., cupric 1 mg., ferric 2 mg., and nickel 1.5 mg. Nitric acid must not exceed 0.1 millimoles and ammonium or sodium nitrate 0.5 millimoles. Cadmium, manganese, lead, and zinc may be present up to 10 mg. without interference.

A small amount of gelatin is added to stabilize the colloidal dispersion of the silver complex. The complex formed with the dihydropyrimidine compound is as sensitive as and more stable than the *p*-dimethylaminobenzalrhodanine compound, especially at higher silver concentrations. If gold and palladium are present, the former reagent gives less intense complexes.

The reaction is not suited for spectrophotometric studies because of the low stability and slow rate of reaction. As little as 0.0002 mg. of silver may be detected by this method.

Procedure. Transfer an aliquot of solution, containing not more than 0.015 mg. of silver, to a 100-ml. volumetric flask. In a similar flask take a portion of standard. Add to each, 10 ml. of a buffer made by adding 320 ml. of 1:16 nitric acid to 200 ml. of 8 per cent solution of anhydrous sodium acetate, and diluting to 1 liter. Add 1 ml. of a 0.2 per cent gelatin solution, and dilute to about 90 ml. Add 0.6 ml. of a 0.01 per cent solution of 2-thio-5-keto-4-carbethoxy-1,1,3-dihydropyrimidine in acetone. This should be not more than a week old. Dilute the contents of the flasks to volume, and mix thoroughly. The color develops within 40-50 minutes and is then ready for balancing. Alternatively, use a series of standards.

Silver by *p*-Dimethylaminobenzalrhodanine

p-Dimethylaminobenzalrhodanine in alcohol [22] or acetone [23] solution is a sensitive reagent for silver, producing a pink to red color.[24] The reagent is sensitive to 1 part of silver in 40 million parts of water.[25] Silver may be determined directly without evaporation in solutions containing 0.06-9 mg. per liter with an accuracy of at least 3 per cent.

Organic solvents such as acetone, ether, alcohol, ethyl acetate, formaldehyde, and acetic acid react to produce color with the reagent. The presence of chlorides in the sample fades the color produced with silver ions, yielding low results. This difficulty may be overcome by repeated

[22] Irl C. Schoonover, *J. Research Natl. Bur. Standards* **15**, 377-84 (1935).

[23] K. Heller and P. Krumholz, *Mikrochemie* **7**, 213-22 (1929).

[24] Fritz Feigl, *Z. anal. Chem.* **74**, 380-6 (1928); I. M. Kolthoff, *J. Am. Chem. Soc.* **52**, 2222-6 (1930).

[25] G. Ettisch and J. Tamchyna, *Mikrochemie* [2] **4**, 92-6 (1931).

evaporations of the solution to dryness with concentrated nitric acid. Gold, platinum, palladinm, cuprous, and mercurous ions react with the reagent to give similar colors.[26] Nitrites produce a deep yellow color with the reagent but are readily removed by acidifying the sample slightly, adding a crystal of sodium azide, and boiling for a few minutes. Small amounts of sulfuric acid or potassium sulfate increase the depth of color. If sodium nitrate or calcium carbonate are present in concentrations greater than 1 mg. per liter, the same effect may be observed. Ammonium nitrate in excess of 0.6 mg. per liter decreases the sensitivity. Beer's law holds over a range of 0.1-1.0 ppm. Small amounts of lead and copper have no effect.

Procedure. Measure out an aliquot of sample containing 0.0006-0.09 mg. of silver and a similar portion of standard. The acidity of the sample must be controlled at about 0.12 *N*. If that of the sample is in doubt, as it usually is, approximately neutralize, and add 0.5 ml. of 1:3 nitric acid. Make a similar adjustment of the standard. Add 1 gram of ammonium acetate to each and dilute to approximately 13 ml.

To sample and standard add 0.5 ml. of a 0.02 per cent solution of *p*-dimethylaminobenzalrhodanine in ethanol, dilute to 15 ml., and shake. The maximum color develops after 30 minutes and does not change for at least an hour. Compare by balancing, or read the transmittance with a green filter.

Miscellaneous

o-Toluidine[27] determines as little as 7.5 mg. of silver per liter by a blue color. Up to 3 times that amount, the average error is 3 per cent. For smaller amounts the error is 10-15 per cent. To a 1-2 ml. aliquot of solution add 1 ml. of an acetate buffer for pH 4, and 0.25 ml. of a 1 per cent solution of *o*-toluidine in ethanol. Mix well, keep at 0-15° to prevent decomposition, and after 15 minutes compare with similarly prepared standards.

Tannin[28] detects 0.008-0.250 mg. of silver as nitrate with an accuracy of ±5 per cent. Acidify an aliquot of sample with nitric acid, and evaporate to dryness in a small porcelain crucible. Transfer with 2 ml. of water to a colorimeter tube. Add 4 ml. of fresh 0.5 per cent filtered

[26] I. M. Kolthoff, *Pharm. Weekblad* 58, 463 (1921).

[27] L. M. Kul'berg and S. B. Serebryanii, *Mem. Inst. Chem. Tech., Acad. Sci. Ukrain.* S. S. R. No. 4, 37-42 (1937).

[28] Const. G. Makris and Raoul Menaché, *Ann. chim. anal. chim. appl.* 22, 117-20 (1940).

tannin solution and 2 drops of a 5 per cent solution of egg white in 0.4 per cent sodium hydroxide solution. Compare after 5 minutes with similarly treated standards.

Under controlled conditions, a red colloidal dispersion of silver chromate may be obtained to determine as little as 0.025 mg. of silver.[29]

Silver is estimated nephelometrically in slightly acid solution as the sulfide[30] by a method analogous to estimation of lead as the sulfide. Iron and lead do not interfere. Gelatin may be added as a protective colloid. The desirable concentrations are 5.4-54 mg. of silver per liter in which range results are generally accurate to 2 per cent.[31] A suitable hydrogen sulfide reagent is prepared from saturated sodium sulfide solution and concentrated hydrochloric acid in place of saturating water with hydrogen sulfide gas.

For determination, make the sample solution faintly ammoniacal, dilute to about 70 ml., and add 10 ml. of clear hydrogen sulfide solution. Acidify with 1:1 nitric acid to dissolve any iron sulfide, and dilute to 100 ml. Compare with a standard similarly prepared.

When a dilute ammoniacal silver solution containing gelatin is treated with sodium hyposulfite,[32] a clear yellow sol forms. It is suitable for colorimetric estimation of silver.[33] The method is applicable to 1-20 mg. per liter. Copper, cobalt, nickel, and cadmium interfere and must be removed before determination of silver.

Prepare an ammoniacal gelatin solution as follows: Soak 2 grams of ash-free gelatin in 100 ml. of water for a few hours. Then heat to dissolve. Add 100 ml. of concentrated ammonium hydroxide, dilute to 1 liter, and hold at 95° for 6-7 hours. If ash-free gelatin is not available, add 0.2 gram of diammonium phosphate to precipitate calcium and magnesium, and filter after precipitation is complete.

Transfer a 10-40 ml. aliquot of the sample to a 50-ml. volumetric flask. Add 1:1 ammonium hydroxide until faintly ammoniacal. Add 10 ml. of the gelatin solution and 0.04 gram of dry sodium hyposulfite.

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[29] H. G. Kraimick, *Mikrochemie* 26, 158-64 (1939).

[30] P. W. Danckwortt, *Arch. Pharm.* 252, 29-76 (1914); Tibor V. Heidlberg, *Biochem. Z.* 192, 238-40 (1928); Arthur F. Scott and Frank H. Hurley, *J. Am. Chem. Soc.* 56, 333-5 (1934); Charles H. Greene, *ibid.* 56, 1269-72 (1934).

[31] Lucia de Brouckère and Robert Petit, *Bull. soc. chim. Belg.* 45, 717-25 (1937).

[32] The term hyposulfite refers to the salts of $H_2S_2O_4$ and is not to be confused with the older term of hyposulfite for salts of $H_2S_2O_3$, commonly known as thiosulfates.

[33] E. E. Jelley, *J. Soc. Chem. Ind.* 51, 191-3T (1932); O. F. Miller, *Chemist-Analyst* 25, No. 1, 8-10 (1936).

Dilute to 50 ml. with 1:6 ammonium hydroxide. Warm to 50° in a water bath to develop the color. Compare with a standard similarly treated.

For approximate determination of amounts of silver of the order of 0.005 mg., coprecipitation as the iodide or the sulfide with barium sulfate serves.[34] The color of a standardized precipitate varies with the amount of silver and estimation is by a series of standards.

Silver in ammoniacal pyridine solution containing dimethylglyoxime reacts with potassium cyanonickelate solution to give a yellow color,[35] which may be read photometrically at 400 mμ. It is applicable over the range 0.002-7 mg. of silver and conforms to Beer's law.

With silver present, the rate of reduction of ceric sulfate in approximately 1:3 hydrochloric acid solution may be followed by visual photometry at 436 mμ.[36]

The reciprocal of the time required for reduction of a given amount of ceric sulfate and the amount of silver present bear a linear relationship. The method is suited to estimation of 0.001-0.01 mg. of silver.

[34] Pierre Sue, *Ann. chim. anal.* 28, 26 (1946).

[35] Sidney Siggia, *Anal. Chem.* 19, 923-4 (1947).

[36] Hidehiro Goto and Takanobu Shiokawa, *J. Chem. Soc.* (Japan) 64, 840-4 (1943).

CHAPTER 4

MERCURY

THE NATURAL occurrence of mercury as the element or the sulfide is unimportant analytically. It is used in varied medicinal preparations and therefore occurs biologically. What is of even greater importance is its occurrence in the air due to appreciable volatility. Like lead, mercury is a cumulative poison and therefore the presence of traces is important.

The methods of preparation of samples for determination of mercury differ from those for the majority of other metals in one important particular. The metal and its salts have appreciable volatility, even at the temperature of boiling water.

Common methods are by dithizone and as the sulfide, but the very fact that current literature contains so many methods is an indication that no one method is as outstanding for mercury as the dithizone method is for lead.

SAMPLES

Air.[1] Absorb mercury vapors in air by washing the requisite volume in chlorine water. The removal of chlorine from such a solution is a problem. If air is passed through, some volatilization of mercuric chloride results. Transfer the solution to a flat-bottom dish and place in a desiccator containing phosphorous pentoxide and soda lime. If the solution is evaporated completely to dryness, some loss of mercuric chloride occurs. Permit the solution to evaporate to about 0.5 ml. Take up in 1 ml. of water and use as sample or dilute to a known volume and use an aliquot.

An alternative solution for absorption is 0.25 per cent of iodine in 3 per cent aqueous potassium iodide.[2] Then determine as the cuprous iodide complex.

Suspended Particles. Use the midget impinger (Figure 3) with the solution of 0.25 per cent of iodine in 3 per cent aqueous potassium

[1] E. V. Alekseevskii, *Zhur. Prikladnoi Khim* 4, 411-4 (1931); Vladimir Majer, *Z. anal. Chem.* 87, 352 6 (1932).

[2] E. C. Barnes, *J. Ind. Hyg. Toxicol.* 28, 257-61 (1946).

iodide. Efficiency in absorption is about 90 per cent. Determine as the cuprous iodide complex.

Fabric. Heat a weighed sample of cloth to 50° with a mixture of 3 parts of concentrated hydrochloric acid, 1 part of concentrated nitric acid, and 8 parts of water, under an air condenser. Pour off the extract

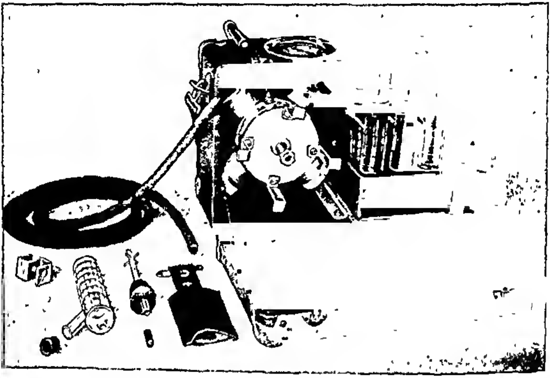

FIG. 3
Midget Impinger. (Mines Safety Equipment Co.)

and repeat the treatment about 6 times. Wash the residue with hot water. Concentrated *aqua regia* must not be used because of the volatility of mercuric chloride with hydrogen chloride. Make the combined extracts and washings slightly alkaline with 10 per cent sodium hydroxide solution and saturate with hydrogen sulfide. Let stand in a warm place, make just acid with acetic acid, and let stand for a time.

Prepare a filter in a carbon tube by depositing a surface of kaolin, a little sodium silicate, and fine quartz on glass wool. Add dilute acid and wash well with boiling water. Use this for separating the precipitate and wash until all chlorides are removed. Treat the precipitate on the filter with warm 1:3 nitric acid and finally with boiling 1:3 nitric acid. The mercury remains on the filter as mercuric sulfide. Dissolve this with

1 ml. of a warm 3:1 mixture of concentrated hydrochloric and nitric acids, dilute, and neutralize with 10 per cent sodium hydroxide solution. Dilute to 10 ml. Loss by volatilization during solution in *aqua regia* must be avoided by care in heating.

Urine. There are varied technics for determination of mercury in urine, an important determination because the body endeavors to rid itself of mercury by excretion.[3] Complete destruction of organic matter without loss of mercury is difficult, complicated by volatility and instability of mercury salts at higher temperatures. Mercury may be distilled as a mercury ammonium chloride complex.

Transfer a suitable sample to the 800-ml. flask of the apparatus shown in Figure 4. Add 5 ml. of concentrated sulfuric acid to minimize foaming, and glass beads. Distill off water to reduce to 30-50 ml., discarding this distillate. Add a gram of copper sulfate and 15-20 grams of ammonium sulfate. Again connect with the special apparatus and put 50 ml. of concentrated sulfuric acid in the dropping funnel. Digest slowly until foaming subsides, then somewhat more rapidly. If carbon carries over due to charring, return the entire distillate to the digestion flask. If lipoidal material distills over, filter it from the distillate.

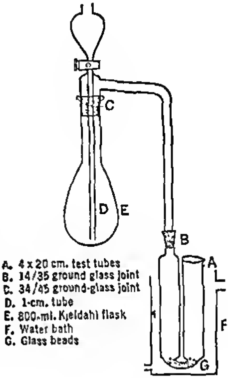

FIG. 4
Special Apparatus for Separation of Mercury

When digestion is complete, fit a chlorine inlet to the funnel and slowly bubble chlorine through the digestate. Heat the flask with a fair-size flame until the mercury is distilled, usually about 20 minutes. Aspirate air to displace chlorine and decompose the bulk of the hypochlorous acid. Transfer the distillate to a separatory funnel, filtering if necessary. Pass sulfur dioxide through this solution for a minute to remove any remaining trace of hypochlorous acid. This sample is suitable for dithizone extraction.

[3] F. L. Kozelka, *Anal. Chem.* 19, 494-6 (1947); cf. Jean L. Monnet, *Bull. soc. chim. biol.* 27, 269-71 (1945).

As another technic[4] add 10 ml. of concentrated sulfuric acid and 10 ml. of concentrated nitric acid to 100 ml. of urine. Heat at 80-100° for 0.5 hour under a reflux and add 1 per cent potassium permanganate solution until in slight excess. Cool and add 30 per cent hydrogen peroxide until the precipitated manganese dioxide is redissolved. Let cool and transfer to a centrifuge tube. Pass hydrogen sulfide through the solution for about 0.5 hour, then air for a similar time to remove excess hydrogen sulfide. Add 3 ml. of saturated aqueous barium chloride solution and centrifuge for 20 minutes. Decant the supernatant layer, dissolve mercury from the barium sulfate precipitate with 1 ml. of concentrated nitric acid, dilute, and filter. Wash the precipitate with water. Add 1:1 ammonium hydroxide to the filtrate and washings until neutral and use as sample.

Alternatively, to a liter of urine containing of the order of 0.5 mg. of mercury[5] add 100 ml. of concentrated sulfuric acid and 20 grams of potassium permanganate. Reflux for an hour to complete oxidation of organic matter and carefully add oxalic acid crystals until the solution is just decolorized. Add 1 ml. of 10 per cent sodium arsenate solution and pass in hydrogen sulfide for 3 minutes. Heat to boiling to coagulate the precipitate of mixed sulfides. Filter and wash the precipitate with water saturated with hydrogen sulfide. Dissolve the precipitate from the filter in 0.4 ml. of concentrated nitric acid and 1.2 ml. of concentrated hydrochloric acid. Dilute nearly to 100 ml., add a gram of hydroxylamine hydrochloride, and dilute to about 100 ml. Use as sample for determination by dithizone.

As another technic,[6] mix 500 ml. of urine, 50 ml. of 30 per cent sodium hydroxide solution, 10 grams of sodium thiosulfate, and 2 ml. of 10 per cent sodium sulfate solution. Add 1 ml. of 20 per cent barium chloride solution dropwise. Heat on a boiling water bath for 1-2 hours and add 0.2 ml. more of the barium chloride solution. Let settle overnight and decant the clear upper layer. The mercury will have been collected on the barium sulfate precipitate by sorption. Collect the precipitate in a centrifuge tube and wash with water. Dry at room temperature by blowing with a stream of dry air.

Add 2-3 drops of bromine to the dry precipitate to convert the mercury to the bromide, and remove all excess bromine with the air stream. Extract the dry precipitate with ether, which will dissolve mercuric

[4] Alberto J. Llacer, *Anales asoc. quím. argentina* 27, 49-63 (1939).
[5] R. Milton and J. L. Hoskins, *Analyst* 72, 6-10 (1947).
[6] R. Fabre and R. Moreau, *Bull. soc. chim. biol.* 26, 202-5 (1944).

bromide. Evaporate the ether extracts and take up the residue in water as a sample. The method will isolate 0.0025 mg. of mercury.

Blood. Place 25 grams of blood in the 800-ml. digestion flask of the apparatus shown in Figure 4. Follow the first technic for urine starting at "Add a gram of copper sulfate . . .".

Tissue. Treat as for blood except add 75 ml. of concentrated sulfuric acid instead of 50 ml.

As another technic,[7] grind a sample of 100-200 grams with water to a semi-liquid. Treat the finely divided material with 10 per cent by weight of potassium chlorate, and 20 per cent by weight of concentrated hydrochloric acid. Heat to destroy the organic matter, passing all gases evolved into water to absorb the mercuric chloride volatilized. Filter and wash the residue on the filter with the liquid from absorption of mercuric chloride. Treat the filtrate with sulfurous acid to reduce excess potassium chlorate and arsenic compounds. Boil a few minutes to remove excess sulfurous acid. Pass a slow current of hydrogen sulfide through the solution for 18 hours and separate the mixed sulfides by filtration.

To separate the mercury, dissolve the mixed sulfides in a mixture of 3 parts of concentrated hydrochloric acid and 1 part of concentrated nitric acid. Evaporate to dryness *in vacuo*. Take up the residue with warm water and filter into a test tube. Dilute to about 20 ml. and add 1 ml. of concentrated hydrochloric acid. Add solid ammonium oxalate until the solution is saturated. Introduce into the solution a piece of pure copper or gold wire of such length that it is not quite covered by the solution. The purity of the copper wire, if used, is of great importance, as any zinc present would distill off with the mercury, vitiating the determination. It may be checked by heating the wire in a glass tube to see whether any sublimate is given off, after which it should be ignited and reduced with methanol vapor. Heat in a flame the upper part of the tube containing the solution and wire, and draw it out until the two sections are connected by a rather narrow neck. Evacuate the tube with a water pump and seal by fusing the neck. This is to exclude air and avoid oxidation of the copper. Heat the sealed tube with its contents for 24-36 hours at 50-60°. Break the neck of the tube, remove the wire on which mercury has been deposited, and wash by placing the

[7] A. Stock and W. Zimmermann, *Z. angew. Chem.* 41, 546-8 (1928); Rudolph Thilenius and Robert Winzer, *ibid.* 42, 284-8 (1929).

wire in several successive portions of water, avoiding stirring. Dry over phosphorous pentoxide for 3 hours at room temperature.

A tube suitable for distillation of the mercury from the wire is one of Pyrex glass; it has a capillary constriction in the middle and is drawn out to a fine capillary at the open end to reduce the movement of air inside. Place the wire in the closed end of the tube and heat for 5 minutes, cooling the second portion of the tube in running water, so that the sublimed mercury will be condensed on the walls. Do not permit the wire to fuse to the glass. The condensed mercury can be seen with a magnifying glass. Cut off the tube at the constriction.

Connect one end of the tube to a suction pump and the other end to a source of chlorine gas dried by passing through sulfuric-acid wash bottles. Pass chlorine through until the air has been substantially all replaced by chlorine. Fuse the tips of the capillaries. Put the micro-bomb, now containing mercury and chlorine, into a suitable holder and heat throughout the entire length at 250° for 15 minutes. The mercury is changed to mercuric chloride. Move the tube so that one of the capillaries projects from the holder. While cooling this part in water, heat the rest of the bomb for 3 hours at 250-300°. The mercuric chloride sublimes, and condenses in the exposed part. Let the tube cool, open the capillary containing no salt, connect to the suction pump, and evacuate. Allow the tube to fill with air. Repeat the evacuation several times to remove all free chlorine. Heat the tip of the closed capillary so that the mercuric chloride is sublimed away from the extreme tip and cut this off. Dissolve the contents of the tube in water and use as sample, or dilute to a known volume and use an aliquot.

Organic Materials.[8] Reflux an amount of sample, which will vary in amount according to the mercury content, with concentrated nitric acid to which a suitable volume of water has been added, also according to the nature of the sample. When ashing is nearly complete, add saturated aqueous potassium permanganate solution in small volumes, the acid being diluted at least 1:5 with water at that step. When oxidation is complete, cool and add 30 per cent hydrogen peroxide solution, a few drops at a time, until the precipitated manganese dioxide has been dissolved. This may also be reduced by additions of solid potassium

[8] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, pp. 471-2, Association of Official Agricultural Chemists, Washington, D. C. (1945).

nitrite.[9] Finally add 0.5 gram of hydroxylamine sulfate. If antimony is present, add 15 ml. of saturated aqueous tartaric acid free from mercury. Use this solution as sample for determination by dithizone.

Alternatively,[10] transfer a sample of food or biological material containing 0.01-0.1 mg. of mercury to a Kjeldahl flask. Add 30 ml. of a mixture of equal volumes of concentrated sulfuric and nitric acids, more if the sample appears to require it. Heat gently so as to avoid vigorous reaction and excessive foaming. Finally heat for 2 hours at full flame. Let cool somewhat and add a small amount of concentrated nitric acid occasionally if necessary to remove charred material. Fatty acids may remain undigested. Let cool, filter, and dilute to 250 ml.

Transfer a 25-ml. aliquot to a 250-ml. separatory funnel for separation of the mercury from other metals. Add 50 ml. of 1:75 hydrochloric acid and 5 ml. of a 20 per cent solution of hydroxylamine hydrochloride. Mix well and add 10 ml. of a solution containing 5.5 mg. of dithizone per liter of chloroform (page 3). Shake for a minute and let stand to separate. Withdraw the chloroform layer and repeat the extraction with 10 ml. more of the dithizone solution. Combine the extracts which now contain the mercury and discard the aqueous layer. Wash the chloroform extracts with 50 ml. of 1:70 hydrochloric acid and discard the washings.

Add 50 ml. of 1:70 hydrochloric acid and 5 ml. of 40 per cent potassium bromide solution. Shake vigorously for a minute. Any copper present remains in the dithizone phase but mercury is transferred to the aqueous phase. Discard the chloroform layer and wash the aqueous layer with 10 ml. of chloroform.

Prepare a buffer solution containing 150 grams of anhydrous disodium phosphate and 38 grams of anhydrous potassium carbonate per liter. Add 10 ml. of this to buffer the sample at about pH 6.0. For the dithizone method this solution is not to be acidified with sulfuric acid.

Separation of mercury by coprecipitation with copper as the sulfide is also used.[11] Dissolve the filtered sulfides in chlorine water and remove excess chlorine to give the sample solution. Precipitation of the mercury for concentration, as by addition of finely divided zinc to a solution of pH 1-2, is sometimes practiced.[12] Filter the precipitate, dissolve in 1:1 nitric acid, and oxidize with potassium permanganate solution.

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[9] Alexander O. Gettler and Robert A. Lehman, *Am. J. Clin. Path., Tech. Suppl.* 2, 161-4 (1938).

[10] Edwin Lang and K. W. Nelson, *J. Assoc Official Agr. Chem.* 25, 399-403 (1942).

[11] J. F. Reith and C. P. van Dijk, *Chem. Weekblad* 37, 186 93 (1940).

[12] W. O. Winkler, *J. Assoc. Official Agr. Chem.* 21, 220 8 (1938).

Standard

Dissolve 0.1354 gram of mercuric chloride in water and dilute to 1 liter. This contains 0.1 mg. of mercury per ml. For 0.01 mg. per ml. dilute 10 ml. of this solution to 100 ml. When a standard free from chlorides is required use 0.1000 gram of mercury and dissolve in the minimum volume of 1:3 nitric acid. Dilute to 1 liter and make further dilutions as previously outlined.

Mercury by Dithizone

Mercuric compounds react readily with dithizone[13] in solutions of a pH in the range of 1-2 to give a yellow to orange color.[14] The extraction of mercury dithizonate occurs readily at any level above pH 1.0. Mineral acid is used and since such an amount of chloride would interfere, it is essential that nearly all of the acidity be due to sulfuric acid and that more than a small amount of chlorides be absent. The keto complex formed is insoluble in water but soluble in chloroform or carbon tetrachloride. Reaction is by 1 part by weight of mercury with 2.6 parts of dithizone. The mixed color is preferable to conversion to free dithizone. Copper, silver, gold, palladium, and bivalent platinum also form colors with dithizone in acid solution. Silver must not exceed the amount of chloride present. No more than a trace of bismuth is permissible. On exposure to daylight the solution develops a dirty green tinge[15] and in sunlight becomes bluish purple. In the dark this photochemical effect is reversed and the original color restored. The presence of acetic acid inhibits the color change. Separation from a small amount of copper is possible in the procedure, but inconvenient. Other than in very high concentrations, other metals forming dithizonates do not interfere.

A solution of dithizone in aqueous tetrasodium pyrophosphate can be used, avoiding extraction but thereby also eliminating concentration of the color in small volume.[16] Elimination of other metals and development of the color at neutrality or in slight alkalinity is probably preferable.[17] Then comparison is with a series of standards.

[13] For a more detailed discussion of this reagent and precautions necessary to it use, refer to page 3.

[14] W. O. Winkler, *J. Assoc. Official Agr. Chem.* 18, 638-44 (1935); *ibid.* 19, 233-6 (1936); H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66-72 (1939); Thomas H. Maren, *J. Lab. Clin. Med.* 28, 1511-14 (1943).

[15] J. F. Reith and K. W. Gerritsma, *Rec. trav. chim.* 64, 41-6 (1945).

[16] R. I. Alekseev, *Zavodskaya Lab.* 7, 415-17 (1938).

[17] Alberto J. Llacer, *Anales asoc. quím. argentina* 27, 49-63 (1939).

Organic matter must be absent from the sample solution as it inhibits the extraction of mercury by dithizone. Accuracy to 0.003-0.005 mg. of mercury is usual and to 0.001 mg. can be attained.

Procedure. Due to sensitivity of the color, work in subdued or artificial light. Take an aliquot of solution of sample to contain 0.01-0.02 mg. of mercury in a separatory funnel. Neutralize with 1:1 ammonium hydroxide, dilute to about 20 ml., and add an equal volume of 1:17 sulfuric acid. Add 5 ml. of a solution containing 10 mg. of dithizone per liter of carbon tetrachloride. A solution in chloroform may be substituted. Shake for about 1 minute and let the solvent separate. If the solution does not show either a green tinge due to excess reagent or a reddish-violet tinge due to reaction of the reagent with a trace of copper present, add another 5 ml. of reagent solution and shake again. If necessary continue to add reagent until a change in color can be detected.

Copper Absent. Draw off the solvent layer and, if not clear, centrifuge in a stoppered tube. Protect from light, which causes a shift in the keto-enol equilibrium, and read the transmittance as promptly as possible. Use a 500 mμ filter to read mercury dithizonate and, if especial care is necessary, check this by determining the excess dithizone, using a 625 mμ filter.

Copper Present.[18] Withdraw the solvent solution and shake with an equal volume of 1.5 per cent sodium thiosulfate solution in 1:35 sulfuric acid. Mercury is extracted into the aqueous phase, leaving copper in the solvent layer which may then be discarded.

Add a 1 per cent solution of potassium permanganate to the aqueous layer until the color remains pink. Destroy excess potassium permanganate with a 10 per cent solution of hydroxylamine hydrochloride. Add an equal volume of 1:17 sulfuric acid and proceed as for copper absent, starting at "Add 5 ml. of a solution containing 10 mg. of dithizone . . ."

Mercury by Di-β-Naphthylthiocarbazone

This is a reagent closely related to dithizone but more sensitive.[19] Its mercury complex is suitable for photometric examination by mixed-

[18] W. O. Winkler, *J. Assoc. Official Agr. Chem.* 21, 220-8 (1938); *ibid.* 22, 341-6 (1939); *ibid.* 23, 310-13 (1940).

[19] I. B. Suprunovich, *J. Gen. Chem.* (U.S.S.R.) 8, 839-43 (1938).

color technics.[20] Precautions similar to those with dithizone methods are necessary (page 3). Reagent blanks are of the order of 0.0005 mg. The color is red with a blue shade contrasted with the yellow given with dithizone. In all cases the maximum is shifted toward the higher wave lengths. Interference by copper, silver, gold, palladium, and platinum is similar to that with dithizone. Accuracy to ±0.0002 mg. is obtainable in the lower ranges.

Procedure. Select a volume of prepared sample solution expected to contain up to 0.05 mg. of mercury. If not already distinctly acid, neutralize with 1:1 sulfuric acid, add 10 ml. in excess, and dilute to about 100 ml. If copper is absent the next step may be omitted.

Copper Separation. Extract by shaking for 1 minute with 5 ml. of chloroform containing 20 mg. of di-β-naphthylthiocarbazone per liter. This is equivalent to about 0.025 mg. of mercury. Add another 5 ml. of reagent solution and shake again. Continue such additions until the color of the extraction medium no longer changes.

Withdraw the organic solvent extract and add successively 75 ml. of water, 2 ml. of 1:1 sulfuric acid, and 4 ml. of 1.5 per cent sodium thiosulfate solution. Shake for 1 minute. The mercury will be transferred to the aqueous layer but the copper will remain in the chloroform layer,[21] which is now discarded. Wash the aqueous layer with three 2-ml. portions of chloroform to remove traces of the reagent, making the last removal of chloroform complete.

Transfer the aqueous layer to a flask and add 5 ml. of saturated potassium permanganate solution. Heat under a reflux for about 10 minutes and let cool. Decolorize by dropwise addition of 5 per cent hydroxylamine hydrochloride solution, and 1 ml. in excess. Again heat to boiling under the reflux, let cool, and dilute to 100 ml.

Mercury Estimation. Extract with reagent solution of a concentration depending on the mercury content. For up to 0.005 mg. of mercury use 10 ml. at 6 mg. per liter, for 0.005-0.025 mg. use 25 ml. at 8 mg. per liter, for 0.025-0.050 mg. use 20 ml. at 20 mg. per liter. Separate the chloroform layer, wash once with water, and read in the photometer in a cell of appropriate length, with a filter of around 515 mμ.

[20] Donald M. Hubbard, *Ind. Eng. Chem., Anal Ed.* 12, 768-71 (1940); Jacob Cholak and Donald M. Hubbard, *ibid.* 18, 149-51 (1946).
[21] W. O. Winkler, *J. Assoc. Official Agr. Chem.* 21, 220-8 (1938).

Mercury by *s*-Diphenylcarbazone

The blue to purple color[22] of the colloidal product of reaction between mercuric ion and *s*-diphenylcarbazone is shown by as little as 10 ppm. of mercury in the test solution. If the semicarbazide is used[23] it is readily oxidized to the semicarbazone, even by air. Potassium *s*-diphenylcarbazone forms cerise to blue salts with zinc, lead, copper, mercury, iron, chromium, nickel, and cobalt. It is therefore essential that the mercury be separated from other heavy metals before developing the color. Small amounts of strong electrolytes interfere by causing flocculation of the colloidal material. The colored complex can be extracted with benzene[24] but the complexes of other ions are also extractable and that technic has not been developed to any substantial extent.

The method can be applied photometrically.[25] The reagent must be present in a ratio greater than 2:1 to the mercury to obtain a constant color which suggests that the product is composed of 2 parts of diphenylcarbazide for each part of mercury.[26] The order of mixing does not affect the rate of color development, which requires about 15 minutes. It does not fade in several hours if undue exposure to oxygen is avoided. Zinc does not interfere up to about 5 times the mercury concentration. Interfering ions are iron, cobalt, nickel, lead, copper, silver, gold, cyanide, and chromate. Minute amounts of bromide and iodide may be present.[27] Chloride ion over 3.5 mg. per liter causes a decomposition into colorless products. Substantial amounts of ammonium ion slightly lessen the color.[28] Electrolytes over 200-400 mg. per liter cause flocculation in less than an hour. In the range 3.5-6.3 the higher the pH the greater the tendency to flocculate. Over pH 7 the reagent is a hydrogen-ion indicator. Below pH 2.6 the solution is decolorized. Urea used as buffer increased this tendency. Adjustment of pH to ±0.3 in the range 3.5-4.5 is adequate. The color deviates somewhat from Beer's law. Over the range 2-15 mg. of mercury per liter the perceptible differ-

[22] M. P. Cazeneuve, *Compt. rend.* 130, 1478 (1900); *Bull. soc. chim.* (3) 23, 492 701 (1900); F. Feigl and F. Neuber, *Z. anal. Chem.* 62, 369-84 (1923); A. W. Scott, *J. Am. Chem. Soc.* 51, 3351-2 (1929).

[23] P. Ménière, *Compt. rend.* 146, 754 6 (1908).

[24] N. Strafford and P. F. Wyatt, *Analyst* 61, 528 35 (1936).

[25] F. W. Laird and Sister Alonzo Smith, *Ind. Eng. Chem., Anal. Ed.* 10, 576-8 (1938).

[26] Fritz Feigl and F. L. Lederer, *Monatsh* 45, 115 32 (1924).

[27] Alfred Stock and Erich Pohland, *Z. angew. Chem.* 39, 791-2 (1926).

[28] *Cf.* V. Majer, *Z. anal. Chem.* 87, 352-6 (1932).

ence is 0.035 mg. per liter which is the normal sensitivity. Not over 0.005 mg. per ml. should be present or flocculation will occur quickly.

Procedure. Prepare the reagent by saturating absolute ethanol with 2-diphenylcarbazide and use it within 24 hours. Store in a glass-stoppered bottle in the dark. Oxygen hastens discoloration. Select a sample free from interfering substances and transfer 10 ml. to a suitable tube. Add acetic acid to pH 4.0 as read by the glass electrode. Add 1 ml. of the reagent and mix well. Let stand for 15 minutes and read photometrically, or by balancing or dilution against a standard of closely similar concentration.

Mercury by Dimethylaminobenzalrhodanine

Mercuric ion gives a brick-red colloidal dispersion with dimethylaminobenzalrhodanine in neutral or acid solution.[29] If the amount of mercury is excessive a precipitate is formed but otherwise the intensity of color is proportional to the mercury content. On long standing it may precipitate. Large excess of the reagent masks the color unless discharged by addition of nitric acid. Such discharge does not destroy the color with mercury, and 0.05 N nitric acid while nearly discharging the color of the reagent permits development of color with mercury. The preferred range is 0.01-0.20 mg. of mercury in 100 ml. of solution. Unreliable results are obtained if the colloid is stabilized with natural gum. Even traces of sulfate or halogen prevent satisfactory formation of the color. Only silver, cuprous, gold, platinum, and palladium ions interfere. The original method calls for electrolytic separation of mercury or platinum and subsequent solution in nitric acid. Cupric ion does not interfere.

Procedure. Select a volume of chloride- and sulfate-free sample to contain 0.01-0.20 mg. of mercury. Adjust to neutrality in a 100-ml. Nessler tube and dilute to about 90 ml. Similarly take suitable volumes of mercuric nitrate standard, to contain about the same amount of mercury as the sample, and dilute to about 90 ml. To each sample and standard add 5 ml. of 1:15 nitric acid. Mix well and to each add 3 ml. of a solution containing 0.03 gram of the reagent in 100 ml. of acetone. Mix and after 5 minutes compare the sample with the series of standards.

[29] N. Strafford and P. F. Wyatt, *Analyst* **61**, 528-35 (1936).

Mercury as the Colloidal Sulfide

As with so many other metals, mercury can be converted to the brown colloidal sulfide for estimation.[30] The method will readily detect 0.01 mg. of mercury in the final 10-ml. volume. Methods of preparation of sample which isolate the elementary mercury or the sulfide and subsequently dissolve it are suitable. Many metals will give a precipitate of the sulfide under the specified conditions.

Procedure. Use 1 ml. of a sample solution which is quite concentrated. Add 1 ml. of slightly ammoniacal 1 per cent gum arabic solution, and 1 ml. of clear saturated hydrogen sulfide solution. Stopper, mix, let stand for 15 minutes, and compare with a series of standards prepared at the same time. These should contain 0.1-1.0 mg. of mercury in 10 ml.

Mercury by Potassium Iodide and Ammonium Hydroxide

When a dilute, nearly neutral solution of mercury is treated with an ammoniacal solution of potassium iodide, a brown color is produced.[31] This is practically a reversal of the Nessler method for ammonia.

Sample and standard must be treated with the same amounts of reagent at the same time and subjected to the same conditions in all respects. Unless the ratio of iodine to mercury lies between 3:1 and 16:1 the reaction is not quantitative. Unless the solution is moderately alkaline no color develops. Excess ammonium chloride or the order of adding the reagents does not affect the results. It is applicable over the range 0.01-1 mg. of mercury.

Procedure. Prepare the reagent by dissolving 0.2 gram of potassium iodide, 6.0 grams of sodium hydroxide, and 2.0 grams of ammonium chloride in water. Dilute to 100 ml. To 10 ml. of standards containing 0.01-0.10 mg. of mercury per ml. and to 10 ml. of sample, add 5 ml. of the reagent. Mix and compare the brown color of the sample with the standards. If necessary make up a more limited series of standards.

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[30] Kohn Abrest, *Ann. chim. anal. chim. appl.* 7, 353-5 (1925); V. A. Pʼyankov and M. L. Loevskil, *J. Applied Chem.* (U.S.S.R.) 9, 2153-4 (1936); Manfred Oesterlin, *Arch. Pharm.* 280, 451-3 (1942).

[31] L. L. Lloyd and W. M. Gardner, *J. Soc. Chem. Ind.* 31, 1109-12 (1912); Louis Jordan and W. P. Barrows, *Ind. Eng. Chem.* 16, 898-901 (1924); E. H. Vogelenzang, *Pharm. Weekblad* 66, 657 (1929). M. Rangier and H. Rabussier, *Compt. rend. soc. biol.* 119, 1072-4 (1935); R. Cambar, *Bull. trav. soc. pharm. Bordeaux* 78, 112-26 (1940).

Mercury as the Cuprous Iodide Complex

Addition of cupric ion, potassium iodide, sulfite, and bicarbonate to a mercury solution results in a pink to orange suspension of the double compound which is sufficiently stable to compare with a series of standards.[32] A suitable sample contains 0.02-0.1 mg. of mercury per 100 ml.

Procedure. Prepare a reagent containing 1 part of 7 per cent cupric chloride, $CuCl_2 \cdot 2H_2O$, solution and 4 parts of 30 per cent sodium sulfite, $Na_2SO_3 \cdot 7H_2O$, solution. Unless already present, add 3 per cent of potassium iodide to the sample, after neutralizing if necessary, and a similar amount to the standards. Add 4 volumes of sample solution and corresponding standards to 3 volumes of the reagent, and mix until the initial precipitate dissolves. Add 6 parts of 8 per cent sodium bicarbonate solution, mix well, and let stand for 10 minutes before comparing.

Miscellaneous

Separated elementary mercury has also been used to reduce phosphomolybdotungstic, phosphomolybdic, or molybdotungstic acid to molybdenum blue.[33] As reagent,[34] heat 34 grams of ammonium molybdate or 25 grams of molybdic anhydride with 300 ml. of 5 per cent sodium hydroxide solution for about 20 minutes. This drives off all the ammonia, which is often present in molybdic acid also. Add 100 grams of sodium tungstate, 50 ml. of 85 per cent phosphoric acid, and 500 ml. of 1:4 hydrochloric acid. Close the flask with an air condenser and heat on a water bath for 4 hours. Cool and dilute to 1 liter. The yellow color should disappear when the reagent is made alkaline in use.

To 5 ml. of sample and a comparable standard add successively 1 ml. of reagent, 1.5 ml. of 20 per cent lithium sulfate solution, and 3 ml. of 20 per cent sodium carbonate solution. Mix well and add 3 ml. more of the sodium carbonate solution. Let stand for 5 minutes and dilute each to the same known volume. Compare by balancing or dilution.

An iodomercurate with strychnine, quinine, or other alkaloids is

[32] N. G. Polejaev, *Hig. Truda* 14, No. 6, 86 (1936); E. Peregud and E. Kuz'mina, *Hig. Truda* 14, 71-2 (1936); *Lab. Prakt. (U.S.S.R.)* 1937, No. 5, 36-9; S. Plisetskaya, *ibid.* 1939, No. 12, 25-7; *Khim. Referat. Zhur.* 1940, No. 6, 61-2; E. C. Barnes, *J. Ind. hyg. Toxicol.* 28, 257-61 (1946).

[33] V. Ciocalten and C. Titel, *Compt. rend. soc. biol.* 112, 621-2 (1933).

[34] Otto Folin and Hsein Wu, *J. Biol. Chem.* 38, 81-110 (1919).

determined nephelometrically at 0.002-0.01 mg. of mercury per ml.[35] The formation of red mercuric iodide on a background of cuprous iodide is another method of comparison.[36] Yet another method, in this case for mercury vapor, is to use it to blacken a coating of selenium sulfide on paper.[37] The degree of blackening is a function of time of exposure, temperature, velocity of the air current over the reagent, and the concentration of mercury. Several elaborate types of apparatus for applying the method quantitatively have been developed. In practical use the reagent is specific for mercury and can detect, in 4 minutes at 70°, 0.25 ppm. of mercury by volume.

Mercury forms a red, insoluble compound with ammonium tetrathiocyano-diammono-chromate, known as Reinecke's salt. When filtered on an inorganic filter and washed it can be dissolved in thiourea and methylethyl ketone, with addition of ethanol or acetone if desired.[38] It is suitable for photometric determination. Copper, silver, gold, thallium, and cadmium must be absent.

[35] J. Golse and M. Jean, *Bull. soc. pharm. Bordeaux* 69, 168-76 (1931); S. I. Sinyakova, *J. Gen. Chem.* (U.S.S.R.) 4, 1081-7 (1934).

[36] G. F. Vagner, *Lab. Prakt.* (U.S.S.R.) 14, 9-10, 22 (1939).

[37] B. W. Nordlander, *Ind. Eng. Chem.* 19, 521 (1927); *Gen. Elec. Rev.* 30, 442 (1927); U. S. Patent 1,711,742 (1929).

[38] C. Mahr, *Angew. Chem.* 53, 257-8 (1944).

CHAPTER 5

COPPER

Copper is an element so widely distributed as to lead to its determination in practically every type of sample. It is found in alloys. It occurs in medicinal products, in biological samples, and in foods. In short, traces of it are almost everywhere.

The outstanding method for the determination of very small amounts is by use of diethyldithiocarbamate. The sensitive dithizone method is also applicable. For larger amounts the color with ammonia or amines serves. For still larger amounts the ASTM has adopted the bromide method. Although copper is one of the metals determined largely with a very few reagents, there are still numerous other reagents used to a lesser extent.

Table 2. Some Comparative Sensitivities of Copper Methods

Form of Copper	*Mg. per Ml.*	*Accuracy in Per Cent*
Sulfate[1]	0.013	25
Sulfate[1]	0.13	3
Pyridine complex[1]	0.0006	25
Pyridine complex[1]	0.013	3
Ammonia complex[1]	0.0006	10
Ammonia complex[1]	0.025	3
Ammonia complex[2]	0.2	1
Ferrocyanide[1]	0.00012	25
Ferrocyanide[1]	0.0018	3
Ferrocyanide[2]	0.0008	1.8
Dimethylglyoxime[1]	0.00003	25
Dimethylglyoxime[1]	0.00012	3
Chloride in concentrated acid[2]	0.008	1.6
Bromide in concentrated acid[2]	0.002	1.5
Sulfide[2]	0.004	5.3
Diethyldithiocarbamate[2]	0.0002	1.8
Dithizone	0.00003	2
Salicylate[2]	0.004	5.3

[1] C. A. Goethals, *Z. anal. Chem.* 104, 170-82 (1936).

[2] Ralph H. Müller and A. T. Burtsell, *Mikrochemie ver. Mikrochim. Acta.* 28, 209-28 (1940).

The relative sensitivity of the methods naturally varies according to the method of preparation of the sample and the reagent used to develop the color. Some comparisons of reagents are available and data are shown in Table 2. Since different instruments are used, such values are naturally purely relative and in some cases differ substantially from values reported by others.

SAMPLES

Magnesium-base Alloys.[3] Weigh a sample of up to 1.5 gram of alloy, such that it will contain 0.1-1.2 mg. of copper and 0.03-0.6 mg. of iron. Add 25 ml. of water and dissolve by adding about 10 ml. of 48 per cent hydrobromic acid in small portions for each gram of sample. Add 3 ml. excess. Transfer to a 50-ml. volumetric flask, dilute to volume with water, and mix. Use aliquots, usually 10 ml., to determine copper and iron, reading in concentrated hydrobromic acid.

Alternatively,[4] weigh 1.0 gram of the alloy with less than 0.5 per cent copper, cover the sample with 40 ml. of water, and add 20 ml. of 1:10 sulfuric acid. Warm if necessary to dissolve the main part of the sample. When reaction ceases add 5 ml. of 3 per cent hydrogen peroxide. Boil this until the residue dissolves to a clear solution. Cool, dilute to 100 ml., and use aliquots.

Aluminum.[5] Digest a 0.5-gram sample with 60 pellets of potassium hydroxide, 1 ml. of 3 per cent hydrogen peroxide, and 20 ml. of water. When reaction is complete, dilute with 10 ml. of water and add 30 ml. of concentrated nitric acid, 20 ml. of 50 per cent tartaric acid, 10 ml. of 85 per cent orthophosphoric acid, and 100 ml. of water. Heat until solution is complete, dilute to a known volume, and use a suitable aliquot as sample.

Alternatively,[6] dilute after reaction with alkali is complete and filter the residue. This contains all the copper and nickel. Dissolve it in nitric acid and dilute as sample solution.

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[3] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 323-5. American Society for Testing Materials (1946).

[4] F. W. Haywood and A. A. R. Wood, "Metallurgical Analysis by Means of the Spekker Absorptiometer," p. 51. Adam Hilger, Ltd., London (1944).

[5] Lothaw-Koninkovsky, *Aluminium* 25, 208-13 (1943).

[6] S. Bertoldi, *Alluminio* 12, 37-9 (1943).

Aluminum Alloys.[7] Dissolve a 5-gram sample in 100 ml. of 10 per cent sulfuric acid. Manganese, iron, and aluminum go into solution but copper remains undissolved unless ferrous iron is allowed to oxidize to ferric. In that case discard the solution and repeat. Filter and dissolve the residue from the filter with 5 ml. of hot 1:1 nitric acid into a 50-ml. flask. Wash the filter with water until the volume of filtrate reaches about 25 ml. or, if the copper is relatively high, dilute to 50 ml. and use an aliquot. For large amounts of copper in aluminum use the ammonia complex, for small amounts use the sodium diethyldithiocarbamate reagent.[8]

Alternatively,[9] prepare mixed acid containing 60 ml. of 1:4 sulfuric acid, 20 ml. of concentrated hydrochloric acid, and 20 ml. of concentrated nitric acid. Dissolve 0.2 gram of sample in 15 ml. of the prepared acid. Evaporate to strong fumes, let cool, and add 60 ml. of water. When the residue is dissolved, let cool and dilute to 250 ml. Use aliquots, usually 10 ml.

In the separation of titanium the copper is displaced with zinc and filtered (page 424). By dissolving this residue in hot 1:1 nitric acid a solution is obtained suitable for estimation of copper.

Aluminum-copper-silicon Alloys.[10] Dissolve 0.1 gram of sample in 5 ml. of 14 per cent sodium hydroxide solution. When there is no further reaction, filter and wash the residue on the filter with 1 per cent sodium hydroxide solution. Reserve the filtrate for determination of silicon.

Dissolve the residue from the paper in a minimum of hot 1:1 nitric acid. Boil the filtrate to oxidize any iron present. Neutralize the filtrate with concentrated ammonium hydroxide and add 10 ml. in excess. Filter into a 100-ml. volumetric flask and wash on the filter with 1:50 ammonium hydroxide until the flask is made up to volume. This is the prepared sample for reading as the ammonia complex.

Aluminum-copper-nickel-manganese-iron Alloys.[11] Treat 0.5 gram of sample with 40 ml. of 20 per cent sodium hydroxide solution. After

[7] Herm. A. J. Stelljes and P. Langer, *Aluminium* **24**, 169-72 (1942); cf. H. Pinsl, *ibid.* **19**, 439-46 (1937); A. Jordy, *ibid.* **21**, 27-31 (1939).

[8] P. Urech, *Helv. Chim. Acta* **22**, 331-4 (1939).

[9] Robert F. Partridge, *Ind. Eng. Chem., Anal. Ed.* **17**, 422-4 (1945); cf. D. F. Phillips and L. L. Edwards, *Metal Ind.* (London) **66**, 409-10 (1945).

[10] A. A. Tikhonova, *Zavodskaya Lab.* **11**, 616-17 (1945).

[11] W. Stross, *Metallurgia* **32**, 257-61 (1945).

the initial vigorous reaction, boil gently for not over 5 minutes. After partially cooling add 50 ml. of 2:1 nitric acid and mix well. Boil for about 3 minutes and cool. Without filtering, transfer to a 100-ml. volumetric flask and dilute to volume as sample for copper, nickel, manganese, iron, and titanium. Use the diethyldithiocarbamate method with an aliquot of 1-2 ml. suitably diluted.

Zinc-aluminum-copper or Zinc-aluminum-iron Alloys.[12] Dissolve a 2-gram sample with a mixture of 20 ml. each of 1:1 nitric acid and 1:1 hydrochloric acid. Evaporate substantially to dryness to drive off excess acid and oxides of nitrogen. Take up in 200 ml. of water and, with cooling, titrate with 33 per cent sodium hydroxide until the zinc and aluminum hydroxides first precipitated are redissolved. Filter the precipitated hydroxides of copper, iron, manganese, and magnesium, using a 2-liter flask as receiver. Set aside this solution for determination of aluminum.

Dissolve the precipitated hydroxides from the filter with 20 ml. of 1:2 nitric acid. Add 1:1 ammonium hydroxide to this solution to precipitate iron and manganese, leaving copper and magnesium in solution. Filter, wash, and dilute this filtrate to a known volume to aliquot for determination of copper and magnesium. Reserve the precipitate for determination of iron and manganese.

Steel and Cast Iron Electrolytically[13] Transfer to a beaker a 5-gram sample containing 0.5 per cent of copper or less, a proportionally smaller sample if the copper content is greater. Add 92 ml. of water and 8 ml. of concentrated sulfuric acid. Cover the beaker with a watch glass and boil until the iron is dissolved The copper is mostly in the residue. Solution of high-chromium and 18-8 steels, or of steels containing over 5 per cent of copper, will be incomplete, in which case add 5 drops of concentrated nitric acid and continue to boil until reaction is complete.

Unless nitric acid has been added, wash down the sides of the beaker and, when the solution is again boiling vigorously, add 5 ml. of a solution containing 100 grams of ferric sulfate and 50 ml. of concentrated sulfuric acid per liter. Continue to boil vigorously for 5 minutes. This reagent oxidizes the copper so that it is now in solution as cupric ion. Transfer a drop to a spot plate and test with 5 per cent sodium thiocyanate solution. A bright, cherry red indicates that oxidation is com-

[12] Erich Bischof and Georg Geuer, *Metall u. Erz*, 38, 57-63 (1941).

[13] William S. Levine and Henry Seaman, *Ind. Eng. Chem., Anal. Ed.* 16, 80-2 (1944).

plete. If the color is only pink add 3 ml. more of reagent, boil for 5 minutes, and repeat the qualitative test. One addition is usually sufficient for carbon steels but cast irons are apt to require more. If 7 per cent or more of molybdenum is present, or if tungston is high, it is better to add concentrated nitric acid 5 drops at a time, as too much ferric sulfate would be required. Usually 15-30 drops of the acid will suffice. Filter into a beaker and wash the residue 5 times with cold water before discarding. In routine work the residue may be left in the beaker. Dilute the solution to about 150 ml. and cool to 15-18°. A high temperature will give a black rather than a bright deposit.

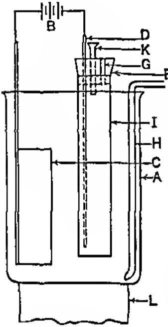

FIG. 5
Apparatus for Electrolytic Deposition of Copper

Apparatus for electrolysis is shown in Figure 5. A is the beaker, usually 200 ml., on a block L. The source of power, B, is a 6-volt, 150-ampere motor generator with the voltage adjusted by a rheostat not shown. A weighed 20-mm. platinum gauze cathode, C, is used. The anode, D, is a platinum wire about 1.0 mm. in diameter and 100 mm. long, passed through a rubber stopper, F, into an alundum thimble, I. A convenient alundum diaphragm is No. 7338, R.A. 360. A notch, G, in the stopper permits the escape of gases evolved at the anode. Sulfuric acid, diluted 1:24, as anolyte is added to the alundum thimble through tube K. Compressed air to stir the main solution enters through the tube H.

Place the beaker under the electrodes and fill the alundum cell to overflowing with 1:24 sulfuric acid, catching the overflow in the sample solution. Raise the beaker to position and put the supporting block in place. Cover the beaker with split watch glasses, turn on the current, and adjust the voltage to 2.1-2.2. Bubble air through H as vigorously as possible without loss of electrolyte. The initial current will be about 0.1 ampere, the final about 0.02 ampere. Below 2.0 volt, copper does not plate out. In the range of 2.1-2.2 a deposit should be noticeable in 1-5 minutes. If too large an excess of ferric ion has been added it will take longer. At best the deposit will be bright. Plain carbon steels and cast iron usually give a dark red color, varying from bright red to nearly black. Alloy irons and steels usually give dull black to blue black.

About 45 minutes will be required for plating out each 0.3 per cent of copper in a 5-gram sample. Completeness of deposition can be tested on a spot plate if chromium does not interfere with a blue color. For this test add 3 drops of concentrated nitric acid to an equal amount of sample and stir until the initial black color disappears. Add 2 drops of 85 per cent orthophosphoric acid and mix. Add 2 drops of 0.2 per cent solution of sodium diethyldithiocarbamate and mix. A brown color disappearing almost immediately on stirring indicates copper is absent. A yellow color, which fades after a few minutes, indicates the presence of copper, in which case retest at 10-minute intervals until it is absent. Since no test is possible with high-chromium steels, electrolyze for 45 minutes after a copper deposit is visible.

When all the copper is deposited, remove the watch glasses, turn off the air and remove the block L. With the current still flowing, lower the beaker slowly, washing the cathode thoroughly to remove iron salts. For plain steels or cast irons this is suitable for gravimetric determination after washing with alcohol and drying. With more than 0.2 per cent of molybdenum present a hydrated molybdenum oxide probably deposits with the copper.

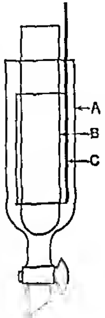

FIG. 6
Apparatus for Stripping of Electrolytic Copper

Figure 6 shows the apparatus for stripping the deposit. An open-top cylindrical separatory funnel, A, has a tube 80 mm. long, with an inside diameter of about 25 mm. An ordinary 16 mm. test tube, B, is cut down to a height of about 120 mm. Partially fill this with water and place in the separatory funnel. Slip the electrode, C, over the test tube and add about 13 ml. of 1:1 nitric acid to the outer space, which should be sufficient to cover the electrode. Leave this acid in contact about 1 minute, moving the electrode up and down occasionally to stir the solution. Open the stopcock and drain the solution into a 50-ml. flask. Wash the electrode with water and drain into the flask. Repeat once more which will give about 40 ml. of solution in the flask.

As recommended for the ammonia method use 1:4 ammonium hydroxide as the wash solution; thus the nitric acid in the sample will have been partially neutralized. By that procedure checks to 0.01 per cent are obtained when copper is below 0.5 per cent. If copper is relatively high, dilute the contents of the flask to 50 ml. and use an aliquot.

Steel.[14] For colorimetric determination of copper in steel, it must be separated from the large amount of iron present.

Tungsten Absent. Heat a 5-gram sample containing 0.005-0.10 per cent of copper with 100 ml. of 1:5 sulfuric acid until the main portion is dissolved. If much chromium or vanadium is present, add a few crystals of potassium chlorate and boil until carbides are decomposed. Dilute to 300-400 ml. and heat to boiling. A precipitate will be present containing some copper. Add 20 ml. of a 50 per cent solution of sodium thiosulfate to precipitate the balance. Boil vigorously for about 5 minutes to coagulate the sulfides. Filter and wash the residue free from iron salts with 1:50 sulfuric acid saturated with hydrogen sulfide. Finally wash with hot water and discard the filtrate.

Transfer the paper and precipitate, which may contain sulfides of molybdenum, vanadium, arsenic, and tin with the copper sulfide, to a crucible. Ignite at 550° Fuse the ash with 3 grams of sodium pyrosulfate. Take up the cooled melt in 25 ml. of 1:9 hydrochloric acid and dilute to about 100 ml. Neutralize with 5 per cent sodium hydroxide solution and add 0.3 ml. excess. Boil for 3 minutes and set aside for 20-30 minutes. Filter and wash the precipitate of copper sulfide, which may be contaminated with iron, antimony, tin, etc., with 0.5 per cent sodium hydroxide solution. Set aside this filtrate for molybdenum determination. Dissolve the precipitate from the filter with 20 ml. of hot 1:3 nitric acid. Wash the paper with water, and discard. Add 10 ml. of 1:1 sulfuric acid to the filtrate and washings and evaporate to fumes of sulfur trioxide. Cool and take up with about 40 ml. of water. Add 1:1 ammonium hydroxide until the copper present has redissolved and heat to boiling. Let the precipitate settle, and filter. Wash the precipitate with hot 1:4 ammonium hydroxide. If there is considerable precipitate so that sorption of copper may be serious, redissolve in 10 ml. of hot 1:10 sulfuric acid and reprecipitate with 1:1 ammonium hydroxide, filtering and washing as before.

Neutralize the filtrate or the combined filtrates with 1:1 sulfuric acid and add 4 ml. in excess. Dilute to about 100 ml., heat to boiling, and saturate with hydrogen sulfide for 20 minutes. Filter and wash the sulfide precipitate on the filter, discarding the filtrate and washings.

Dissolve the sulfide from the filter with the minimum amount of hot

[14] "Sampling and Analysis of Carbon and Alloy Steels, Methods of the Chemists of the Subsidiary Companies of the United States Steel Corporation as Revised to 1937," pp. 116-20. Reinhold Publishing Corp., New York, N. Y. (1938); cf. W. Åström, *Arch. Eisenhuttenw.* 11, 515-16 (1938).

1:1 nitric acid, using not over 10 ml. Add this solution slowly with stirring to 15 ml. of concentrated ammonium hydroxide. Dilute to 25 ml. and filter through an inorganic filter. Wash and dilute to a known volume with 1:1 ammonium hydroxide as a developed sample for reading by the ammonium hydroxide method.

Tungsten Present. Treat a 10-gram sample with 100 ml. of 1:1 hydrochloric acid. Add 25 ml. of 1:1 nitric acid in small increments and boil gently until a bright yellow residue of tungstic acid remains. Dilute to 150 ml. with hot water and digest for 5 minutes. Filter and wash the precipitate with 1:9 hydrochloric acid. Nearly all of the copper is in the filtrate. Reserve the precipitate for later recovery of copper.

Add 25 ml. of 1:1 sulfuric acid to the filtrate and evaporate just to fumes. If not carried far enough the nitric acid will not all be expelled; if carried too far basic chromium sulfate will be formed. Add 100 ml. of water to the cooled residue and filter if necessary. If filtered wash the residue with 1:20 sulfuric acid before discarding. Add 5 grams of tartaric acid to the filtrate and washings, or the clear solution, and neutralize with 1:1 ammonium hydroxide. Add 10 per cent by volume of 1:1 sulfuric acid. Heat just to boiling and pass in hydrogen sulfide for 15 minutes. Digest for 30 minutes, add paper pulp, filter, and wash with 1:100 sulfuric acid saturated with hydrogen sulfide. Discard the filtrate and reserve the precipitate to which another will be added.

Dissolve the precipitated tungstic acid in a few ml. of concentrated ammonium hydroxide and add 5 grams of tartaric acid. Add 1:1 sulfuric acid until the solution is neutral and 10 per cent by volume in excess. Saturate the solution with hydrogen sulfide and filter. Wash the precipitate with 1:100 sulfuric acid saturated with hydrogen sulfide and discard the filtrate and washings. Add the precipitate to that previously obtained.

Complete as in the absence of tungsten starting at "Transfer the paper and precipitate, which may contain sulfides of molybdenum, . . .".

Alternatively,[15] prepare mixed acid by adding 250 ml. of concentrated hydrochloric acid to 600 ml. of 1:4 sulfuric acid. Warm 5 grams of sample with 100 ml. of this acid. When solution is complete, add 0.3 ml. of 48 per cent hydrofluoric acid and boil for several minutes. Dilute to about 300 ml. and heat to gentle boiling. Add 20 ml. of 50 per cent sodium thiosulfate solution in 4-ml. increments. Boil for 15-20 minutes, when the precipitate should be well coagulated. Decant through a filter and wash well with hot water.

[15] Oscar I. Milner, *Ind. Eng. Chem., Anal. Ed.* **18**, 94-6 (1946).

Return the paper to the balance of the precipitate and add 35 ml. of a mixture of 250 ml. of concentrated nitric acid and 80 ml. of 70 per cent perchloric acid. Thoroughly moisten the paper and precipitate and warm until decomposition leaves only yellow beads of free sulfur. Heat more strongly until the nitric acid is evaporated and perchloric acid fumes strongly. Let cool and add 35 ml. of water. Neutralize with 1:1 ammonium hydroxide and add 5 ml. in excess.

If more than 1 per cent of manganese was present in the steel, add 10 ml. of concentrated ammonium hydroxide and 2 grams of ammonium persulfate crystals to the boiling solution.

Boil for only a minute or two, be sure ammonia is still in excess, and let cool. Filter into a 100-ml. volumetric flask. Add 20 ml. of concentrated ammonium hydroxide and wash the precipitate on the paper with 1 per cent ammonium nitrate solution until the dilution is complete. This is a developed sample ready for reading by the ammonium hydroxide method.

If the copper is to be determined as the diethyldithiocarbamate and nickel and cobalt are absent it may be directly extracted from the iron solution.[16] Dissolve a 0.1-gram sample in 15 ml. of mixed acid containing 100 ml. of concentrated sulfuric acid and 80 ml. of 85 per cent orthophosphoric acid per liter. Add 5-7 ml. of 72 per cent perchloric acid and evaporate to fumes. Take up with 50 ml. of water and add 5 ml. of 50 per cent tartaric acid solution. Neutralize by dropwise addition of 40 per cent sodium hydroxide solution. This sample is ready for addition of the reagent and extraction (page 111), starting at "Add 0.5 ml. of a 0.5 per cent solution. . . ."

Alternatively,[17] if iron is not objectionable in the final solution, simply dissolve a 1-gram sample of steel filings in 100 ml. of hot 1:9 sulfuric acid. Add redistilled nitric acid dropwise until the color is a clear yellow. Let cool, transfer to a 250-ml. volumetric flask, and dilute to volume.

Copper-chromium Steel.[18] Weigh a 5-gram sample into a beaker and add 75 ml. of concentrated nitric acid. Heat as may be necessary to complete solution. Let cool and dilute to about 150 ml. Add concentrated ammonium hydroxide until distinctly alkaline to litmus and heat to boiling. Filter and wash on the filter with 1:4 ammonium hydroxide until the washings are colorless. Set the filtrate aside and transfer the

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[16] M. Jean, *Bull. soc. chim.* **12**, 437-45 (1945).

[17] G. H. Bendix and Doris Grabenstetter, *Ind. Eng. Chem., Anal. Ed.* **15**, 649-52 (1943).

[18] O. V. Datsenko, *Zavodskaya Lab.* **6**, 1402-5 (1937).

precipitate to the original beaker with water. Redissolve by dropwise addition of concentrated sulfuric acid. Reprecipitate, filter, and wash as before, using the same receiver. Evaporate the combined filtrates, and dilute to a known volume.

Ferrous Alloys.[19] For alloys containing up to 1 per cent of copper weigh out 0.5 gram of the sample, reducing this for higher copper contents. Add 20 ml. of a mixture of 150 ml. of concentrated sulfuric acid and 150 ml. of orthophosphoric acid diluted to a liter with water. Heat until disintegration is complete. Add 5 ml. of 1:1 nitric acid and boil until nitrous fumes are driven off. If necessary, heat to fumes to decompose carbides. Finally let cool to room temperature, take up with water, and dilute to 100 ml. for the use of aliquots.

Alternatively,[20] dissolve 0.1 gram of millings or drillings in 10 ml. of 1:2 nitric acid. When solution is complete add 15 ml. of 4 per cent ammonium persulfate solution and boil for a minute. Cool and add 10 ml. of 35 per cent citric acid solution and 10 ml. of concentrated ammonium hydroxide. The diethyldithiocarbamate method is recommended.

Ferrotitanium.[21] To 80 ml. of 1:10 sulfuric acid add a coil of aluminum wire and a 0.25-gram sample. Boil until disintegration is complete and filter out the precipitated copper. Dissolve the well-washed crateipitate in hot 1:1 nitric acid and use all or an aliquot as sample for about 50 ml., of the blue color with ammonium hydroxide.
water and cool
to acidify. If aloys.[22] Dissolve a 0.2-gram sample in 3-4 ml. of 1:1 nitric cipitate the filtil the reaction is complete and nitrous fumes expelled. Dilute to a add about 25 ml. of water. Add 5 ml. of an ammonium olution containing 500 grams of citric acid and 500 ml. of con-Tated ammonium hydroxide per liter. Filter the solution if meta-acid and acid is present, and dilute to 100 ml. Use a suitable aliquot as sample.

ler.[23] Transfer a 1-gram sample to a casserole and add 10 ml. of

W. Haywood and A. A. R. Wood, "Metallurgical Analysis by Means of Absorptiometer," p. 51. Adam Hilger, Ltd., London (1944).

Thomas, *Proc. Am. Soc. Testing Materials* 44, 769-78 (1944).

Barkov, *Zavodskaya Lab.* 12, 546 9 (1946).

[22] F. W. Haywood and A. A. R. Wood, "Metallurgical Analysis by the Spekker Absorptiometer," p. 76. Adam Hilger, Ltd., London (1944).

[23] G. H. Bendix and Doris Grabenstetter, *Ind. Eng. Chem., Anal. Ed.* 15, 649-52 (1943).

concentrated hydrochloric acid saturated with bromine. If needed add a drop or two of bromine. When reaction is complete boil off the bromine. Cool and dilute with about 10 ml. of water. Chill to below 10° and filter off the lead chloride, using a 250-ml. volumetric flask as receiver. Wash the residue on the paper with 5 ml. of cold 1:1 hydrochloric acid and dilute to volume with water.

Copper Ores and Mattes.[24] For samples containing less than 5 per cent of copper, weigh out 1 gram; for 5-10 per cent, use 0.5 gram, and for more than 10 per cent, use 0.25 gram. Transfer to a casserole and cover with a watch glass. Add 10 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric ocid. Heat on a hot plate until reaction has ceased and let cool. Remove the watch glass and slowly add 5 ml. of concentrated sulfuric acid. Evaporate the contents to dense white fumes, keeping the casserole in constant motion. Alternatively heat by an electrio evaporating cone above the casserole, in which case it is not necessary to agitate. Let cool, carefully add 25 ml. of water, and warm until the copper is in solution. Lead os sulfate is insoluble.

Applied to the ammonia method where interfering metals are present, and using a spectrophotometer, this is accurate to ±0.10 per cent of values by the iodide method and duplicable to about ±0.05 per cent.

Raw Ores.[25] Weigh out a suitable sample of the finely powd... ore, according to copper content. Add 3 ml. of concentrote... and 1 ml. of concentrated hydrobromic acid. Heat to ... until the sample is completely disintegrated and reaction... plete. Let settle and filter by decantation into a 100-... flask. Wash the residue thoroughly with hot water and filt... is ony doubt of the complete extraction of the copper, treat the ... with another but smaller amount of the same reagents. Finally ... the filter and residue and dilute the filtrate to volume.

Roasted Ores. Use the preceding method but heat the sample with 5 ml. of concentrated hydrochloric acid until all colored oxides have been dissolved.

Lead Carbonate, Lead Oxide, and Pig Lead. Weigh 30 grams of refined or 10 grams of crude product, finely divided, and add small

[24] J. P. Mehlig, *ibid.* 7, 387-9 (1935); A. T. Shoshin and B. N. Ranskii, *Zavodskaya Lab.* 8, 1054-6 (1939); *Khim. Referat. Zhur.* 1940, No. 4, 54 (1939).

[25] Anders Ringborn, *Metall u. Erz* 40, 228-40 (1943).

portions of hot 1:1 nitric acid until the sample is dissolved. If basic lead nitrate forms, dilute slightly with warm water and boil. Add 30 ml. of 1:1 sulfuric acid and chill in ice. Decant the supernatant liquid through coarse filter paper, and wash by decantation with ice-cold 1:100 sulfuric acid. Discard the paper and residue. Neutralize the filtrate with 1:1 ammonium hydroxide and add 3-4 ml. in excess. Boil a short time and filter. Wash the precipitate with 1:100 ammonium hydroxide. Render the filtrate just acid with 1:1 hydrochloric acid, chill, and filter through a compact filter paper into a 50-ml. volumetric flask. Dilute to volume and, in use of an aliquot, bear in mind that some lead is still present. This is also suitable for estimation of iron.

Red Lead. Treat 30 grams of finely divided sample with 40 ml. of 1:1 nitric acid and slowly add 30-40 ml. of a 3 per cent solution of hydrogen peroxide, stirring constantly. Dry sodium sulfite may be used in place of hydrogen peroxide. Boil until dissolved; then proceed as with lead carbonate, starting at "Add 30 ml. of 1:1 sulfuric acid and chill in ice."

Slag. Weigh out a sample of 0.5-3 grams according to copper content, and add 10 ml. of concentrated hydrochloric acid and 2 ml. of concentrated nitric acid. Heat on a water bath for a few minutes. Dilute to about 50 ml., render slightly ammoniacal, and filter. Wash with hot water and cool. Add just sufficient 1:1 hydrochloric acid to the filtrate to acidify. If a high degree of accuracy is sought, redissolve and reprecipitate the filtered hydroxides. In that case combine the two filtrates. Dilute to a known volume such as 50 ml.

Tailings. Heat 1 gram of sample with 5 ml. of concentrated nitric acid and 0.5 gram of potassium chloride for 0.5 hour. Treat as for slag starting at "Dilute to about 50 ml. . . ."

Silicate Rocks.[26] Follow the preparation of sample for determination of lead (page 10) up to the state where the copper is left in the carbon tetrachloride solution but lead and zinc have been extracted with 1:500 hydrochloric acid.

Evaporate the carbon tetrachloride solution to dryness in Pyrex. Add 1 ml. of concentrated sulfuric acid and 0.2 ml. of 70 per cent perchloric acid to the residue. Heat at 200-250° until colorless. Cool, take up with 10 ml. of water, and add 1:1 ammonium hydroxide until neutral to

[26] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* **9**, 464-9 (1937).

methyl orange. Add 1 drop of 1:5 sulfuric acid to acidify and dilute to 25 ml. for the use of aliquots.

Soil.[27] Weigh a 25-gram sample and add 250 ml. of 1:10 hydrochloric acid. Reflux on a boiling water bath for 3 hours, shaking frequently. Filter the hot solution into a 1-liter volumetric flask and wash the residue with a generous volume of hot 1:10 hydrochloric acid, first by decantation, later on the filter. Dilute to volume with water and use an aliquot.

Alternatively,[28] to a suitable sample add 15 ml. of concentrated hydrochloric acid and an equal volume of concentrated nitric acid. Heat to boiling and after a few minutes let cool somewhat. Add 4 ml. of 1:1 sulfuric acid and again heat to boiling. If necessary to oxidize organic matter, add a few ml. of 30 per cent hydrogen peroxide from time to time. Finally, after all organic matter has been destroyed, heat nearly to sulfur trioxide fumes and let cool. Add about 25 ml. of water and mix well. Filter into a 50-ml. volumetric flask and wash the residue on the filter until the flask is nearly full. Dilute to volume and use an aliquot.

Red Phosphorus.[29] Treat a 2.5-gram sample with 100 ml. of 1:1 nitric acid. Warm gently to start the reaction and then let the vigorous reaction proceed unassisted until complete. When the reaction is over, evaporate until no more brown fumes are evolved. Add about 5 ml. of concentrated nitric acid and heat again until brown fumes cease. Repeat additions of nitric acid and heating until brown fumes are no longer given off. The phosphorus is now present as orthophosphoric acid or one of the poly acids. Evaporate to a sirup and, if the sirup is colored, add about 0.2 gram of potassium chlorate and reheat to decolorize. Cool and add about 20 ml. of water. Heat to boiling to drive off chlorine and oxides of nitrogen. If the solution is not colorless after boiling for 10 minutes, re-evaporate to a sirup and again treat with potassium chlorate. Use the final colorless solution as sample, or dilute to a known volume and take an aliquot. Use the method by sodium diethyldithiocarbamate.

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[27] Erich Stolze, *Bodenk. u. Pflanzenernähr.* **1**, 115-32 (1936).
[28] S. S. Korol, *Pedology* (U.S.S.R.) **1940**, No. 3, 126-32.
[29] J. A. Brabson, O. A. Schaeffer, Anthony Truchau, and LaVerne Deal, *Ind. Eng. Chem., Anal. Ed.* **18**, 554-6 (1946).

Water.[30] Copper can be completely collected from water by coagulation of aluminum hydroxide. To 1 liter of the water add 300 ml. of a per cent aqueous solution of aluminum sulfate and mix well. Let stand until precipitation occurs, or if the water is not alkaline, as in the case of some ground waters, carefully add a few ml. of 10 per cent sodium hydroxide solution with vigorous stirring. Filter the precipitate, wash well, and dissolve from the filter with 4 ml. of 1:4 sulfuric acid. Use the solution as sample or dilute to a known volume and use aliquots.

Alternatively,[31] evaporate 1 liter of water to about 75 ml. and wash into a platinum dish. Add 2 ml. of 1:1 sulfuric acid or enough to neutralize the alkalinity. If much organic matter or clay is present add 5 ml. of the acid. Deposit copper from the solution electrolytically. Dissolve in 3 ml. of 1:3 nitric acid and evaporate to dryness on the water bath. If silver is present add a few drops of 1:1 hydrochloric acid before evaporating. Take up with water and either use as sample or dilute to a known volume and use an aliquot.

For decolorization of highly colored natural waters[32] treat with aluminum sulfate and adjust the pH to 4.1-4.3 for precipitation. There is loss of copper by precipitation below 3.9 or over 5.0. Excess iron and aluminum cause loss of copper if precipitated as hydroxides but are removed by excess of Rochelle salt without loss of copper.

Water and Salt Solutions. A sample was treated under lead (page 16) to yield an acid solution containing the copper, iron, and zinc. Boil that solution to evaporate the ethanol. Dilute to 200 ml. and add 1 gram of ammonium chloride. Heat to boiling, saturate with hydrogen sulfide, and boil to coagulate. Cover the sample and let stand for about 2 hours or until the supernatant liquid becomes clear. Filter, and wash the precipitated copper sulfide without intermission with water saturated with hydrogen sulfide. Reserve the filtrate which contains the iron and zinc

Dissolve the precipitate of copper sulfide in hot 1:3 nitric acid. Cool, add a few drops of phenolphthalein indicator, and make the solution slightly alkaline with 1:1 ammonium hydroxide added dropwise. Add 10 ml. of a 10 per cent ammonium nitrate solution and adjust the volume to 100 ml Boil gently until a test with red litmus paper shows the solution to be neutral. Filter the solution to remove any additional iron that may be precipitated and adjust the volume of the filtrate to

[30] V. T. Chulko, *J. Applied Chem.* (U.S.S.R.) 9, 1898 1900 (1936).
[31] L. de Brouckère and S. Solowiejesky, *Bull. soc. chim. Belg.* 43, 597-625 (1934).
[32] M. Golubeva, *Gigiena i Sanit.* 11, No 5, 29 33 (1946).

100 ml. Use an aliquot for determination by the ferrocyanide metbod. A typical copper content is that in the Baltic Sea which is 0.0015-0.0078 mg. per liter.[33]

Nickel-plating Bath.[34] With the pH adjusted to 1.3-2.3 this bath can be used directly as sample for dithizone extraction of copper.

Organic Samples. There are several well recognized methods for decompositiou of the organic matter to make the ash available. Each has its place.

Dry Ashing. The size of sample used will depend on the expected copper content. Transfer a suitable sample to a silica dish. If tbe sample is liquid add sufficient 1:1 sulfuric acid to make it definitely acid. Evaporate to dryness or in the case of moist solids dry the mass. Then heat over a low burner or on a hot plate until the material chars. Let cool and moisten the charred mass with 1:1 nitric acid. Occasioually a 20 per cent solution of magnesium nitrate is used for this purpose, particularly if the ash content is very low. Again evaporate to dryness, and transfer to a muffle furnace. Raise the temperature to about 500° in the course of about 3 hours and continue to heat at that temperature until the ash is white. If the sample offers difficulty in ignition at this point, remove it from the furnace and repeat starting at "Let cool and moisten . . .".

When ashing is complete and the dish cooled, dissolve the ash in a 5-ml. portion of 1:1 hydrochloric acid. If necessary for complete solution add concentrated hydrochloric acid to this. Finally add water amounting to about twice the volume of acid added. If an insoluble residue remains, filter on a small paper and wash on the paper with 1:4 hydrochloric acid.

Asb the paper and, depending on the amount of ash, treat with suitable volumes of hydrofluoric and perchloric acids, finally adding the aqueous solution of the residue to the main solution. Dilute the final sample solution to a known volume in a volumetric flask and take suitable aliquots.

Magnesium Nitrate Ashing.[35] Transfer 2.5 grams of sample to a porcelain crucible and add 1 ml. of a 50 per cent solution of magnesium

[33] Kurt Buch, *Finska Kemistsamfundets Medd.* 53, 25-37 (1944).
[34] B. B. Knapp, *Proc. Am. Electroplaters' Soc.* June 1944, 109-12.
[35] Ed. Lasausse and L. Frocraine, *J. pharm. chim.* [8] 23, 77-82 (1936).

nitrate. Evaporate and ash, finally heating to a dull red. Dissolve the residue in 5 ml. of 1:5 hydrochloric acid. Add 1:1 ammonium hydroxide until the original green is changed to blue. Add 10 ml. of 10 per cent ammonium carbonate solution. Let stand a few minutes and filter out the precipitated iron, aluminum, calcium, and magnesium. Wash the filter with 5 ml. of 1:10 ammonium hydroxide, added in several portions. The precipitate will not have sorbed sufficient copper to justify resolution and reprecipitation and can be discarded. Dilute the filtrate to 100 ml. and use aliquots for reading by the ammonium hydroxide method.

Wet-ashing with Nitric and Sulfuric Acids. Transfer a suitable sample such as 5-ml. of blood, 5 grams of wet tissue or food, or 1 gram of dry tissue or food to a small Kjeldahl flask. If the sample is not already damp, moisten with water. Now add 5 ml. of concentrated nitric acid and 2 ml. of concentrated sulfuric acid. Heat over a direct flame and as charring appears add 1 ml. of concentrated nitric acid from time to time. Continue until no further carbon is visible and then evaporate to fumes of sulfur trioxide. At this stage a colorless or very pale yellow liquid should result. Let cool and add a few drops of water and about 10 ml. of 3 per cent hydrogen peroxide to destroy nitrosyl sulfuric acid. Heat to fumes of sulfur trioxide and again let cool. Take up in water, transfer to a volumetric flask, and dilute to volume for the use of aliquots.

Wet-ashing with Sulfuric, Perchloric and Nitric Acids.[36] Place the sample in a Kjeldahl flask. Close the flask with a funnel to prevent contamination. Add sufficient water to moisten the sample thoroughly and follow with 15 ml. of concentrated sulfuric acid. Heat until the material in the flask turns black and sulfur trioxide fumes are given off. Let cool. Add not more than 5 ml. of 20 per cent perchloric acid. The amount of sulfuric acid present when this is added must not be less than 5 ml. Add 2 ml. of fuming nitric acid. Heat with a small flame until no more oxides of nitrogen are given off. Increase the heat until sulfur trioxide fumes are evolved. Let cool. If the solution is not colorless repeat the addition of perchloric acid and nitric acid and subsequent heating. The final volume will be less than the amount of sulfuric acid originally added as some is reduced to sulfur dioxide and water by the organic matter.

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[36] Stefan Ansbacher, Roe E. Remington and F. Barlow Culp, *Ind. Eng. Chem., Anal. Ed.* 3, 314-20 (1931).

Dilute with water until the sulfuric acid content is less than 15 per cent by volume. Heat to boiling and add a few drops of concentrated nitric acid. The nitric acid will decompose some of the hydrogen sulfide in the next step and give a sulfur precipitate to collect the copper sulfide, and will prevent a suspension of colloidal copper sulfide from forming.

Pass hydrogen sulfide through the solution until it is cold. Let the precipitate settle and then filter through an inorganic crucible with a porous bottom. Wash with 1 per cent acetic acid saturated with hydrogen sulfide until the washings give no test for iron. The copper sulfide must not be exposed to the air for any extended period of time. Place the crucible on a glass triangle over a glass crystallizing dish. Set this on a boiling water bath and add 1 ml. of hot concentrated nitric acid to the crucible. When no liquid remains in the crucible, wash with 2 ml. of hot water. Let stand on the water bath until the solution has evaporated to dryness. Place on a hot plate until no more acid fumes are given off. Avoid raising the temperature so high that decomposition of copper nitrate occurs. Add water to take up the residue, transfer to a volumetric flask, and dilute to a known volume according to the probable copper content indicated by the color.

Wine.[37] Select a volume of sample according to the probable copper content. Acidify and precipitate the copper as sulfide. Filter and dry the sulfide residue. Heat the residue in a covered crucible with concentrated nitric acid until oxides of nitrogen cease to be evolved. Remove the cover and evaporate to dryness. Acidify with 1:1 hydrochloric acid and again evaporate to dryness. Dissolve in water and add a few drops of glacial acetic acid. If the iron content is high add 1 ml. of 10 per cent tartaric acid solution. If necessary, dilute to a known volume in order to use an aliquot.

Whiskey and High Wines.[38] Transfer a 200-ml. sample to a platinum dish and evaporate to dryness on a steam bath. Ignite in a muffle at a barely perceptible redness. This should ash to a grey fluff in 30 minutes. Let cool and add 2 ml. of concentrated hydrochloric acid. Warm on a steam bath to dissolve and transfer to a 50-ml. flask. For the diethyldithiocarbamate method take 20 ml. as sample solution.

Residue from Spirits. Ash the residue left in the flask from dis-

[37] J. Golse, *Bull. soc. pharm. Bordeaux* 71, 24-30 (1933).

[38] Louis Gerber, Ralph I. Claassen and C. S. Boruff, *Ind. Eng. Chem., Anal. Ed.* 14, 364-6 (1942).

tillation of alcohol from spirits. Moisten the ash with a mixture of equal volumes of concentrated nitric and sulfuric acids, evaporate to dryness, and take up with 10 ml. of 1 per cent acetic acid. Filter into a 50-ml. flask and dilute to volume, or use the entire filtrate as sample.

Beer and Wort.[39] Dry ashing is used to obviate the high blanks associated with the large amounts of reagents used in wet ashing. Measure 100 ml. of beer into a clean silica evaporating dish. Add 5 ml. of 1:6 sulfuric acid and evaporate to dryness. Char the residue and finally ignite in a muffle at 500-550° to a fluffy, white, unfused ash. Let cool and add 2 ml. of concentrated hydrochloric acid and 1 ml. of concentrated nitric acid. Evaporate to dryness on a steam bath. Should any carbon be found on so dissolving, return to the muffle and reignite until carbon-free. In that event redissolve and re-evaporate. Add 1 ml. of 1:6 sulfuric acid and transfer with hot water as sample, or dilute to a known volume and use an aliquot. Alternatively, use an aliquot as prepared for determination of lead (page 26).

Biological Samples.[40] Use 10 grams of liver, 20 grams of spleen, kidney or lung, 20 ml. of blood, or 5-20 grams of feces. Saturate a 20 per cent magnesium nitrate solution with magnesium carbonate. Add 5 ml. of this to the sample in a silica dish and ignite until carbon-free. Digest the ash with 5 ml. of concentrated nitric acid for 30 minutes. Add 5 ml. of water and filter into a 50-ml. volumetric flask. Wash the residue with water and then dilute to volume. In general use 0.5-5.0 ml. aliquots from liver, 5-10 ml. from kidney, and 20 ml. from the other samples.

Blood.[41] Measure 0.5 ml. of blood into a 25-ml. tube. Add about 0.25 ml. of concentrated sulfuric acid and 4 drops of 70 per cent perchloric acid. Boil vigorously and, if necessary, add a few drops more of perchloric acid to get no more than a light yellow color. Heat until fumes appear and cool. Add 6.5 ml. of water, then 2 ml. of a solution containing 4 grams of tetrasodium pyrophosphate in 100 ml. of 50 per cent potassium carbonate. Use the entire solution as sample.

Alternatively,[42] transfer 1 ml. of blood to a micro-Kjeldahl flask. Add 2 ml. of concentrated sulfuric acid and heat, first on a sand bath,

[39] Irwin Stone, *ibid.* 14, 479-81 (1942).
[40] P. leRoux van Niekerk, *Onderstepoort. J. Vet. Sci. Animal Ind.* 9, 623-8 (1937).
[41] L. Braun and L. Scheffer, *Biochem. Z.* 304, 397-403 (1940).
[42] Uichiro Sarata, *Japan J. Med. Sci. II. Biochem.* 2, 247-75 (1933).

then over a micro burner, until almost transparent. Let the slightly brown solution cool for a few seconds and add 5-10 drops of 30 per cent hydrogen peroxide. Heat until dense white fumes are evolved. Boil gently for 10-15 minutes, even if all color has disappeared. If necessary, add more hydrogen peroxide and repeat.

To separate the copper, dilute to 30 ml. and transfer to a conical centrifuge tube. Add 0.5 ml. of 1 per cent magnesium chloride solution. Then add 40 per cent sodium hydroxide solution, drop by drop, until a turbidity persists. Add 1:18 sulfuric acid, drop by drop, to remove this turbidity. Add 2 ml. of the sulfuric acid in excess and mix. Pass washed hydrogen sulfide in through a fine capillary at the rate of 20-30 bubbles per minute for 40 minutes. Centrifuge for 20 minutes at 2500 rpm. Decant off the supernatant liquid and add 10 ml. of distilled water saturated with hydrogen sulfide. Stir the precipitate well with a glass rod to wash it, and centrifuge. Decant and wash twice more. Add 2 ml. of 1:2 nitric acid to dissolve the precipitate and heat in a boiling water bath for 15 minutes. Filter through a wet filter paper and wash well. Use this filtrate and washings as sample, or dilute to a known volume and pipet out a suitable aliquot. Normally the copper content of blood is 0.12-0.16 mg. per 100 ml.

Serum.[43] Mix 2 ml. of 1:2 hydrochloric acid with 4 ml. of serum and let stand for 10 minutes. Add 2 ml. of 20 per cent trichloroacetic acid, mix again, and let stand for 10 minutes. Filter and wash the precipitate with 4 ml. of 10 per cent trichloroacetic acid. Discard the residue. Pipet out 6 ml. of filtrate, which represents 3 ml. of serum. To this add 8 ml. of 95 per cent ethanol and concentrated ammonium hydroxide, dropwise, until just on the alkaline side for use as sample. Conventional deproteinizing with trichloroacetic acid recovers about 75 per cent of the copper in the centrifugate but three washings of the residue with 10 per cent trichloroacetic acid increases the recovery to about 97 per cent.[44]

Alternatively[45] evaporate a suitable sample to dryness and ash in an electric furnace for 8 hours. Add 1 ml. of concentrated nitric acid and evaporate to dryness. Take up with 1:20 hydrochloric acid and dilute to a suitable volume for taking aliquots. The average as determined by

[43] H. A. Schmidt, *Biochem. Z.* **302**, 256-61 (1939).

[44] George E. Cartwright, Patricia J. Jones and Maxwell M. Wintrobe, *J. Biol. Chem.* **160**, 593-600 (1945).

[45] Adolph Sachs, Victor E. Levine, Alfred C. Andersen and Agnes Schmit, *J. Lab. Clin. Med.* **26**, 734-9 (1941).

the diethyldithiocarbomate method with isoamyl alcohol extraction is 0.105 mg. per 100 ml.

Organs.[46] Weigh the sample into a 25-ml. tube and dry in a high vacuum. Digest with sulfuric and perchloric acids in a ratio of about 5:1. Cool, dilute, and make just alkaline to litmus with 1:2 ammonium hydroxide. Add 5 ml. of 4 per cent tetrasodium pyrophosphate solution and heat for 30 minutes at 80° to precipitate iron completely.[47] Filter and diluto the filtrate to a known volume for the use of aliquots.

Liver.[48] Dry about 1 gram of liver at 80° and powder. Weigh the dried sample into a platinum crucible, add a few drops of concentrated sulfuric acid, and ash. Dissolve the ash in about 5 ml. of 1:50 sulfuric acid. For moximum accuracy electrolyze this solution for separation of copper, redissolve from the electrode in a few drops of 1:1 nitric acid, and evoporate to dryness. Dissolve the residue with a few drops of 1 per cent acetic acid, transfer to a 10-ml. volumetric flask, and diluto to volume. With lesser accuracy, dilute the solution of ash in sulfuric acid. The solution can be expected to contain 0.0005-0.0025 mg. of copper per ml.

Foodstuffs.[49] To obtain a sample of 0.02-0.1 mg. of copper it is often necessary to use 50-200 grams of dried plant moteriol in contrast to 10 grams of biological products. The presence of more than 10 times as much iron as copper may cause low results.

Weigh 50-200 grams of oven-dried material into a silica dish and ash at 600-850° in an electric furnace. Stir with a stiff platinum wire from time to time until all carbon is removed. Let cool, add 5 ml. of 1:1 hydrochloric acid, and cover. Digest on a hot plate for 30 minutes, then dilute with 10 ml. of water and filter. Wash on the filter and discard any insoluble residue Add 1:1 ammonium hydroxide to the filtrate and boil to coagulate iron and aluminum hydroxides. Filter and set the filtrate aside, without washing the precipitate. Dissolve the hydroxides from the filter with 5 ml. of 1:1 hydrochloric acid and wash the paper once. Reprecipitate as before and this time wash the precipitate with about 10 ml. of 1:100 ammonium hydroxide. Combine the filtrates and

[46] B. Eisler, K. G. Rosdahl and H. Theorell, *Biochem. Z.* 285, 76-7 (1936).

[47] Cf. Alfred Eden and Henry H. Green, *Biochem. J.* 34, 1202-8 (1940).

[48] K. Hinsberg and H. Gockel, *Biochem. Z.* 289, 57-66 (1936).

[49] Jackson B. Hester, *Chemist Analyst* 25, 78-9, 83 (1936); *cf.* John H. High, *Analyst* 72, 60-2 (1947).

boil until the volume is less than 50 ml. and free ammonia is substantially absent. Cool, transfer to a 50-ml. volumetric flask and dilute to volume; or, if copper must be isolated, use the method starting in the second paragraph hereafter.

As an alternative method of ashing,[50] transfer 20-50 grams of a well-disintegrated sample to a 500-ml. flask and heat until excess moisture has been driven off and the material has just begun to char. Let cool and add 10 ml. of concentrated sulfuric acid. Heat until sulfur trioxide fumes are being given off and add concentrated nitric acid, drop by drop, until the contents of the flask are pale straw to colorless. Let cool and add redistilled water to about 50 ml. Again heat until sulfur trioxide fumes appear, thus insuring removal of oxides of nitrogen. Let cool, dilute with water, and transfer to a 250-ml. volumetric flask. Dilute to volume for the use of aliquots; or, if copper must be isolated, use the method starting in the next paragraph.

As a method [51] of isolation of copper, add to the solution diluted to about 50 ml. 2 grams of citric acid and 1 ml. of 1 per cent ferrous sulfate solution, the latter to serve as a collector. Cool and adjust the pH to approximately 8.0 by addition of 1:1 ammonium hydroxide. Cool and pass in hydrogen sulfide for about 20 minutes. Filter on a small paper and wash with dilute ammonium sulfide solution prepared by rendering a saturated aqueous solution of hydrogen sulfide definitely alkaline with 1:1 ammonium hydroxide. All the ammonium citrate must be washed out. Reserve the filtrate for determination of tin.

Dissolve the black sulfide precipitate by gently warming the precipitate and paper in 20 ml. of 1:10 nitric acid. When the black sulfides are completely dissolved remove the paper from the solution and replace in the funnel. Pour the solution through this filter and wash with hot water until the volume of solution reaches about 50 ml. Add 1 gram of ammonium sulfate and heat nearly to boiling. Add 15 ml. of 1:1 ammonium hydroxide, mix well, and keep near boiling until coagulation of ferric hydroxide is complete. Do not boil, as loss of ammonia can result in material sorption of the copper.[52] Filter and wash the precipitate with hot 1:15 ammonium hydroxide until the washings show no color. Reserve the precipitate on the paper for determination of bismuth and lead by methods provided under lead (page 23).

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[50] G. H. Bendix and Doris Grabenstetter, *Ind. Eng. Chem., Anal. Ed.* 15, 649-52 (1943).

[51] J. Hubert Hamence, *Analyst* 62, 18 23 (1937).

[52] J. Hubert Hamence, *Trans. Faraday Soc.* 30, 299-303 (1934); cf. T. Cockburn and Magnus Herd, *Analyst* 63, 482-6 (1938).

Dilute the filtrate to 100 ml. and use an aliquot as sample solution. Zinc may be present in this filtrate with the copper.

Alternatively, in the separation of cobalt (page 357), a dithizone layer containing the copper in chloroform is separated and may be used for estimation of copper.

Milk.[53] Evaporate 250 ml. of milk to dryness and ash in a quartz dish at the lowest possible temperature. The large amounts of phosphates prevent direct extraction of the copper compound. Take up the ash with 25 ml. of 1:4 hydrochloric acid. Add 2 drops of concentrated nitric acid, heat to boiling, and saturate with hydrogen sulfide. Let stand until cold and filter. Wash the filter with 50 ml. of 1:100 acetic acid saturated with hydrogen sulfide.

Ash the paper with the precipitate. Dissolve the ash in 2 ml. of concentrated nitric acid and evaporate to dryness on a water bath. Take up the residue with 10 ml. of 1:3 hydrochloric acid by warming, filter, and wash. Use the filtrate and washings as sample or dilute to a known volume and use an aliquot.

The calcium and phosphate may be precipitated.[54] Wet ash (page 93) and dilute. Render the solution alkaline with a liberal excess of 1:1 ammonium hydroxide to precipitate tricalcium phosphate. When this is complete, filter and evaporate the filtrate to dryness. Take up the residue in 2 ml. of 1:1 hydrochloric acid and again evaporate to dryness. Take up with a few ml. of water as sample. The copper may also be recovered from a diluted wet ash solution by extraction with dithizone in chloroform,[55] thus getting a good separation from iron, tin, aluminum, lead, zinc, nickel, and manganese.

As another alternative,[56] treat a 5-gram sample of milk, condensed milk, cream, butter, etc., with 2 ml. of a 10 per cent solution of ammonium thiocyanate in methanol. Add 1 ml. of 1:4 acetic acid. Mix well and dilute with 15 ml. of methanol. Warm to 40-45°, shake vigorously and let stand for 5 minutes. Again shake and then chill in cracked ice to solidify the fatty layer. Centrifuge and separate the solvent layer which will now have extracted all of the copper. Use a suitable aliquot as sample for determination by the addition of guaiac.

The usual copper content is around 0.12-0.15 ppm. unless grossly con-

[53] C. A. Elvehjem and C. W. Lindow, *J. Biol. Chem.* 81, 435-43 (1929).
[54] Josef Krenn, *Mikrochemie* 23, 149 59 (1937)
[55] N. D. Sylvester and L. H. Lampitt, *Analyst* 60, 376-82 (1935).
[56] G. Schwarz and O. Fischer, *Molkerei-Ztg.* (Hildesheim) 54, 345-6 (1940).

taminated. The presence of copper or iron promotes deterioration of milk products.[57] Gravimetric methods are only applicable for over 5 mg.

Milk Powder.[58] Reflux the sample with a solution of potassium thiocyanate in 95 per cent acetone. Filter, dilute to a known volume, and use an aliquot for determination by diethyldithiocarbamate.

Yeast. Take a yeast suspension containing about 0.5 gram of yeast. For pressed yeast wash 2-3 grams successively with water, alcohol, and ether to obtain a powdery solid on drying and grinding. Of the latter use 0.5 gram. Add 5 ml. of 50 per cent ammonium nitrate solution to the sample in a silica dish and evaporate to dryness. Char thoroughly, then ignite in a muffle at not over 500° and below the fusion temperature of the ash. Remove from the muffle when partially ashed, let cool, and add 5 ml. of water. Heat on a water bath to extract soluble salts and filter on a small paper. Wash the filter with several small portions of hot water. Set the filtrate aside and ignite the paper in the original dish as before except that the temperature may be increased somewhat and ashing is to be complete. Let the dish containing the white ash cool and add the reserved filtrate. Evaporate to dryness and add 1 ml. of concentrated nitric acid and 2 ml. of concentrated hydrochloric acid. Evaporate to dryness and let cool. Add 1 ml. of 1:6 sulfuric acid and transfer with hot water as sample, or dilute to a known volume and use an aliquot.

Food Coloring.[59] Wet ash as for any organic material (page 93), take up in water and dilute to a concentration of not over 5 per cent acid. Pass in hydrogen sulfide for about 30 minutes, let stand for about 30 minutes, and filter. Wash the filter well and discard the filtrate. Dissolve the sulfides of copper and lead from the paper in 2 ml. of hot 1:1 nitric acid, using an evaporating dish as receiver. Wash the paper well and discard. Add 1 ml. of concentrated sulfuric acid and evaporate the filtrate to fumes of sulfur trioxide. Let cool, take up the residue in 10 ml. of chilled 50 per cent ethanol, and let stand in cold water for an hour. Filter and wash the paper with 4 ml. of 50 per cent ethanol. Discard the lead sulfate on the paper.

To remove iron, render the filtrate definitely alkaline and boil to

[57] G. Schwarz and O. Fischer, *Proc. 11th World's Dairy Congr., Berlin* **2**, 52-6 (1937).

[58] M. Boulet and W. D. McFarlane, *Can. J. Research* **23B**, 70-5 (1945).

[59] T. Macara, *Analyst* **64**, 339-43 (1939).

coagulate the hydroxide. Finally filter and wash the filter with a few ml. of water. Add 1:1 hydrochloric acid to the filtrate until the precipitate of copper hydroxide first formed is fully redissolved. Dilute to a known volume and use suitable aliqnots.

Gelatin.[60] Weigh 20-50 grams of gelatin in a tared platinum or porcelain dish of about 150-ml. capacity. Ash in a furnace previously heated to 500-550°. When cool, moisten the ash with water, add about 5 ml. of concentrated hydrochloric acid, and filter if necessary. If filtered, ignite the residue, dissolve in 5 ml. of 1:1 hydrochloric acid and join with the filtrate. Evaporate to dryness and add 8 ml. of 1:1 hydrochloric acid. Heat to boiling, filter, and dilute to about 40 ml. Heat nearly to boiling, saturate with hydrogen sulfide, and let stand in a warm place for 0.5 hour. Filter and wash the precipitate thoroughly with warm 1:20 hydrochloric acid saturated with hydrogen sulfide. Transfer the paper and precipitate to a porcelain crucible and ignite in the furnace at 500°. When cool, moisten the ash with 2 ml. of hot, concentrated nitric acid, add about 2 ml. of water, and evaporate to dryness on a steam bath. Dissolve in 10 ml. of 10 per cent ammonium nitrate solution and filter into a 50-ml. flask. In preparation of standards allow for the amount of ammonium nitrate in the aliquot used.

Legumes. Digest about 2 grams of desiccated substances in a Kjeldahl flask with a 1:1 mixture of concentrated nitric and sulfuric acids. When decomposition is complete, evaporate until no more sulfur trioxide fumes are produced. Take up with water and dilute to about 10 per cent acid. A residue of calcium sulfate is left. To oxidize iron add a few drops of 1:1 nitric acid. Precipitate iron and aluminum with a slight excess of 1:1 ammonium hydroxide, filter, and render the filtrate just acid with a few drops of 1:10 sulfuric acid. Evaporate to dryness and calcine slowly in the muffle furnace. When no more ammonia or sulfur trioxide vapors come off, take up the residue with distilled water and add a few drops of 1:360 sulfuric acid to dissolve the copper oxide formed. Dilute to a known volume and use aliquots.

Plant Tissue. A solution containing the copper and cobalt in carbon tetrachloride by dithizone extraction from the ash was set aside in isolation of lead (page 31). Add 5 ml. of 60 per cent perchloric acid to this carbon tetrachloride phase and boil over a low flame to appear-

[60] R. M. Mehurin, *Ind. Eng. Chem.* **15**, 942-3 (1923); E. H. Berry, *J. Assoc. Official Agr. Chem.* **9**, 458-9 (1926); R. M. Mehurin, *ibid.* **14**, 522-5 (1931).

ance of fumes of perchloric acid. Cover with a watch glass and digest until clear. Remove the watch glass and evaporate to dryness. Let cool and dissolve in 12.5 ml. of 0.77 per cent citric acid, which will usually have contained heavy metals to be removed by dithizone extraction (page 3). Dilute this solution to 25 ml. for determination of copper and cobalt on aliquots.

Organic Samples, Particularly Medicinals. The preparation up to the point where copper and arsenic had been extracted in chloroform with a carbamate extraction solution was given under lead (page 28). Add 1 ml. of 1:20 sulfuric acid to the chloroform extract and shake for 5 seconds. This is to remove any trace of entrained phosphate which would later react as arsenic. Draw off the chloroform into a 10-ml. cylinder. Wash the acid with 0.5 ml. of chloroform and discard this wash acid. Dilute the chloroform extracts and washings to 9 ml. with chloroform and transfer to a 50-ml. flask. Wash the cylinder with 1 ml. of chloroform, thus giving a total of 10 ml. in the flask. Swirl gently with 0.5-1.0 gram of anhydrous sodium sulfate until the chloroform solution is clear.

The extract is a developed sample ready for reading the copper. The solution blank for adjustment of the spectrophotometer is 7 ml. of carbamate extraction reagent and 3 ml. of chloroform.

Iron-copper-arsenic Ampoules.[61] Dissolve the contents of the ampoule in 1:3 hydrochloric acid and saturate the solution with hydrogen sulfide. When precipitation is complete, filter and discard the filtrate. Dissolve the precipitate from the paper with 2 ml. of hot 1:1 nitric acid, using a silica evaporating dish as receiver. Evaporate the filtrate to dryness and take up in 2 ml. of 1:1 hydrochloric acid. Filter, if necessary, and use as sample, or dilute to a known volume and take an aliquot.

Organic Chemicals.[62] This preparation of sample is designed for dyes, intermediates, and rubber chemicals. Weigh a 5-gram sample into a 500-ml. Kjeldahl flask. Add 20 ml. of concentrated sulfuric acid and a couple of glass beads. Boil gently until charring and disintegration are complete. This usually requires 15-20 minutes. If the volume is reduced significantly, add 5 ml. portions of concentrated sulfuric acid

[61] Roland A. Bosee and Paul Fehder, *J. Am. Pharm. Assoc.* 29, 141-2 (1940).
[62] G. F. Palfrey, R. H. Hobart, A. F. Benning and I. W. Dobratz, *Ind. Eng. Chem., Anal. Ed.* 12, 94 (1940).

as necessary. When the sample is fully charred let the flask cool and add 5 ml. of fuming nitric acid in several portions. The reaction may be vigorous but is controlled by swirling the flask until reaction subsides. When the addition is complete, heat over a low flame until brown fumes have disappeared. Then boil vigorously for a few minutes and cool. Add another 5 ml. of fuming nitric acid in several portions and repeat the boiling as before. Continue to add nitric acid in this way until it no longer reduces the color, usually three 5-ml. portions.

Cool and add 100 ml. of water, with agitation. Heat to vigorous boiling, preferably with provision for drawing off the vapors, until strong fumes of sulfur trioxide are given off. This hydrolyses all the nitrosyl sulfuric acid and drives off the oxides of nitrogen. Any yellow color present is either iron or undigested organic matter.

To remove such yellow color, if due to organic matter, add 5 ml. of 30 per cent hydrogen peroxide. Heat again until strong fumes of sulfur trioxide are given off and continue to boil for a few minutes. Cool and repeat until no further decrease in color occurs. Two treatments will usually suffice. Cool and dilute the contents with 100 ml. of water. Evaporate to fumes of sulfur trioxide to remove hydrogen peroxide.

Cool and dilute with about 100 ml. of water. If clear, transfer to a 250-ml. volumetric flask. If not clear, heat to boiling, filter into the flask, and wash the filter well. Dilute to volume, mix well, and use aliquots.

Glue.[63] Weigh 10 grams of sample into a small Kjeldahl flask. Add 50 ml. of concentrated sulfuric acid and digest over a small flame until the solution is colorless or, at most, faintly yellow. At this time evolution of sulfur trioxide fumes will have ceased. Let cool and dilute the residue to about 200 ml. Add 5 ml. of saturated mercuric chloride solution and pass in hydrogen sulfide for an hour. Let the precipitate settle, and decant the solution through a filter. Add hydrogen sulfide to 1:100 sulfuric acid and use it for transfer of the precipitate to the paper and for washing on the filter. Reserve the filtrate for later determination of iron.

Dissolve the precipitate from the paper by careful addition of 2 ml. of hot 1:1 nitric acid using a 50-ml. volumetric flask as receiver. Wash the paper carefully and dilute the filtrate to volume for the use of aliquots.

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[63] W. Simon, *Kunstdunger u. Leim* 33, 293-4 (1936).

Tanning Extracts.[64] Select a sample to contain at least 0.1 mg. of copper. For tan liquor from a suspender this requires 100 ml. For chestnut or oakwood liquid extracts, abaut 5 grams are necessary; for other liquid extracts, about 20 grams. A sample of solid extract should be about half that specified for a liquid extract. Due to the presence of zinc in some extracts the ferrocyanide method is not applicable.

Take the sample to dryness in platinum and heat gently until only a swollen mass of carbon remains. Add 5 ml. of concentrated sulfuric acid and continue with the addition af small amounts of concentrated nitric acid until most of the carbonaceous matter is burned out. Add 1:1 hydrochloric acid and evaporate to dryness on a steam bath. Repeat that operation and then dissolve in 5 ml. of 1:1 hydrochloric acid. Dilute to 50 ml. with distilled water.

Add sufficient concentrated ammonium hydroxide to render the solution alkaline. Boil for a few minutes to coagulate iron and aluminum hydroxides. Filter and wash the precipitate with 1:10 ammonium hydroxide. Reserve the precipitate on the paper for later estimation of iron. Boil the filtrate until ammonia is no longer given off and dilute in a volumetric flask to 50 ml., or use the entire sample.

Fertilizer.[65] Digest a sample containing the requisite amount of copper with 2 parts of concentrated nitric acid and 1 part of concentrated sulfuric acid, adding more concentrated nitric acid as necessary to complete the decomposition. Finally, when all organic matter has disappeared, heat until all nitric acid is vaporized and copious sulfur trioxide fumes are emitted. Let cool, dilute with water in excess, boil, and filter. Wash on the filter, further dilute as necessary, and pass in hydrogen sulfide until the copper is completely precipitated as copper sulfide. Filter and save the filtrate for determination of zinc.

Transfer the filter paper carrying copper sulfide to a small flask and add an excess of a mixture of 1 part of concentrated sulfuric acid and 2 parts of concentrated nitrio acid to destroy the organic matter. Heat to boiling and, if destruction of organic matter is incomplete, cool and add 30 per cent hydrogen peroxide solution. Finally, when organic matter is destroyed, heat to copious sulfur trioxide fumes. Dilute with water to about 40 ml. as the sample. Preferably determine by the ammonium hydroxide method.

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[64] M. P. Balfe and H. Phillips, *J. Intern. Soc. Leather Trades Chem.* 15, 226-35 (1931).

[65] W. Y. Gary, *J. Assoc. Official Agr. Chem.* 24, 305-17 (1941); *ibid.* 25, 352-63 (1942).

Fruit-spray Residues.[66] Wash the fruit thoroughly with hot 1:10 nitric acid. To this add sulfuric acid and boil until all organic matter is destroyed. Let cool and dilute if necessary. To this solution add concentrated ammonium hydroxide until it is neutralized and 10 ml. excess is present. Boil for a few minutes, let stand for 30 minutes, and filter out iron and aluminum hydroxides. Either use as sample or dilute to a known volume and take an aliquot.

Rubberized Fabrics.[67] Weigh out a 10-gram sample of fabric, or more according to the copper content. Boil successively with nitrobenzene and benzene in excess until the coating is completely removed. Evaporate the solvents from the fabric and, after again weighing, transfer the fabric to a porcelain dish. Ash carefully, avoiding letting the sample flame at any time.

Dissolve the ash in 10 ml. of 1:1 hydrochloric acid and dilute to about 30 ml. Filter any insoluble residue and discard. Pass in hydrogen sulfide until the precipitate settles. If difficulty is encountered in this, add 0.2 ml. of concentrated nitric acid to give a precipitate of sulfur to help settle out the copper sulfide. Filter and wash with 1:10 hydrochloric acid saturated with hydrogen sulfide.

Dissolve the precipitate from the filter with 2 ml. of hot, 1:1 nitric acid. Dilute to 10 ml., add 1 ml. of concentrated sulfuric acid, and evaporate to sulfur trioxide fumes. Take up the residue and dilute to 50 ml. in a volumetric flask.

Good results have been reported by the ammonia and the ferrocyanide methods on this determination. Calculate results to both the original fabric and the weight after removal of coating although in terms of amounts of copper and manganese, the latter are more significant.

Mildew-proof Fabrics.[68] The preceding method, omitting removal of rubber, is applicable, bearing in mind that copper may run 0.3-6.0 per cent. A suitable method is by ammonium hydroxide using a 1-gram sample.

Textiles.[69] Digest a 10-20 gram sample with 25 ml. of concentrated

[66] D. E. H. Frear, *Ind. Eng. Chem., Anal. Ed.* 11, 494-5 (1939).

[67] A. Rulbing, *Kautschuk* 14, 210-12 (1938).

[68] Carroll L. Hoffpauir and Robert T. O'Connor, *Am. Dyestuff Reptr.* 31, 395-8 (1942).

[69] Raphael E. Rupp, *Proc. Am. Assoc. Textile Chem. Colorists* 1930, 215-7; *Am. Dyestuff Reptr.* 19, 581-3 (1930).

nitric acid, 25 ml of concentrated sulfuric acid, and 5.0 grams of potassium sulfate in a Kjeldahl flask. When digestion is complete, dilute with water to 75 ml., and neutralize with concentrated ammonium hydroxide. Add 3-4 ml. in excess. Boil a few minutes and filter. Wash the precipitate with warm water and discard. Render the filtrate and washings faintly acid with 1:1 hydrochloric acid for use as sample.

Alternatively,[70] put a 10-gram sample in a Kjeldahl flask with 2.5 ml. of fuming nitric acid, 2.5 grams of sodium sulfate, and 2.5 grams of potassium sulfate. When reaction ceases add 13 ml. of concentrated sulfuric acid and heat to complete the digestion. Dissolve the residue in water and add 1:1 ammonium hydroxide until just acid to Congo red. Add 5 ml. of 10 per cent orthophosphoric acid, dilute to 200 ml., and use a suitable aliquot. Electrolytic separation of the copper from the wet ashing solution is suitable,[71] with solution of the deposit and colorimetric determination.

Lubricating Oil.[72] Transfer 10 grams of homogenized sample to a porcelain dish and heat until the contents ignite and burn readily. Remove the flaming dish to a hot plate and maintain at such a temperature that only ash and carbon remain after burning ceases. If liquid or tarry material remain, heat until smoking ceases. Ignite until the carbon is burned away, but not at over 850°.

Treat the residue with 5 ml. of concentrated hydrochloric acid and digest at a low temperature on a hot plate until the soluble ash is dissolved, and filter. If the residue shows dark material, dry, ignite in the same dish, digest with 5 ml. of concentrated hydrochloric acid, and add to the previous filtrate. Discard any remaining insoluble material, regardless of color. Transfer the filtrate or combined filtrate to a 50-ml. volumetric flask, dilute to volume and mix well. This is a sample for copper and for iron.

Separation of Copper, Zinc, Bismuth, Lead, and Tin. Details of the separation are given under lead (page 33). The solution as so prepared contains the copper and zinc, free from the other metals.

Separation from Iron.[73] Acidify a suitable volume of the solu-

[70] Wm. C. Smith, *Proc. Am. Assoc. Textile Chem. Colorists*, 1930, 217-9; *Am. Dyestuff Reptr.* **19**, 583-5 (1930).

[71] Werner Hiltner, *Z. anal. Chem.* **110**, 241-51 (1937).

[72] Louis Lykken, K. R. Fitzsimmons, S. A. Tibbetts and Gerard Wyld, Petroleum Refiner (1945). A.S.T.M. Methods D810-44T and D811-44T.

[73] A. Castiglioni, *Z. anal. Chem.* **97**, 270-3 (1934).

tion with a definite excess of 1:1 hydrochloric acid. Add 0.5 gram of sodium hyposulfite, $Na_2S_2O_4$. Boil the solution for 5 minutes. A precipitate of mixed metallic copper and copper sulfide is obtained. Filter and wash the precipitate on the paper. Dissolve the precipitate with the minimum possible amount of hot 1:1 nitric acid. Cool and dilute to 25-30 ml. Add 1:1 ammonium hydroxide until the solution is just ammoniacal and use as sample, or dilute to a known volume and use an aliquot.

Removal of Iron as Fluoride.[74] Large amounts of iron can be removed from sample solutions as double fluorides such as $5NaF \cdot 2FeF_3$, or $2KF \cdot FeF_3 \cdot H_2O$, or $11KF \cdot 4FeF_3 \cdot 12H_2O$. Adjust the acidity of the solution to contain about 1 ml. of concentrated sulfuric acid. Heat to boiling and add sodium fluoride in small portions until the color due to iron has disappeared due to formation of the undissociated ferric fluoride, FeF_3. A white precipitate of the double fluoride separates. Filter while hot and wash the precipitate on the filter with 1:100 sulfuric acid.

Standards

Prepare a standard solution by dissolving 0.5 gram of pure copper in 10 ml. of 1:1 nitric acid. Dilute the solution to 500 ml. As an alternative, dissolve 1.9645 grams of copper sulfate, $CuSO_4 \cdot 5H_2O$, in water and dilute to 500 ml. One ml. of either of these solutions is equivalent to 1 mg. of copper. The selection between these standards is to be made according to the acid used for solution of the sample. In general the sample will have been heated to sulfur trioxide fumes, in which case the sulfate standard is used. For use in various methods dilute 10 ml. to 100 ml. to get 0.1 mg. per ml., or 10 ml. to 1 liter to get 0.01 mg. per ml.

A standard recommended[75] for nomographic determination by the mixed color dithizone method contains 0.1000 gram of electrolytic copper dissolved in 1:5 nitric acid and diluted to 1 liter with water. For use pipet 10 ml. into a 1-liter volumetric flask, add 8.9 ml. of concentrated hydrochloric acid, and dilute to 1 liter.

Copper by Sodium Diethyldithiocarbamate

Sodium diethyldithiocarbamate by reaction in acid, neutral, or alkaline solution gives the copper salt of diethyldithiocarbamic acid, which

[74] I. V. Tananaev and E. N. Deichman, *Zavodskaya Lab.* 12, 30-7 (1946).
[75] Ernest A. Brown, *Ind. Eng. Chem., Anal. Ed.* 18, 493-5 (1946).

has a golden brown color.[76] Formation of this is one of the most sensitive methods for copper.[77] Since the colored compound is only very slightly soluble in water, the technics in aqueous media usually stabilize a colloidal suspension, as with gum acacia. The pH has little effect on the color over the range 5.7-9.2. For increased sensitivity, the color can be extracted with insoluble organic solvents.[78] The usual solvents are amyl alcohol, amyl acetate, carbon tetrachloride, and bromobenzene.

Cyanides interfere due to the presence of the complex ion. Most metals other than calcium and magnesium may interfere if present in sufficient amount. Ferric iron gives a dark brown precipitate in acid or neutral medium unless ammonium citrate is present at a pH not below 9. In such a buffered solution, nickel gives a greenish yellow, cobalt a dull green, and bismuth a yellow precipitate. The color intensities are not over 20 per cent of that of copper. If separation from large amounts of cobalt and nickel is necessary, precipitation as the sulfide is recommended with lead added as a collector.[79] Small amounts of cobalt and nickel do not interfere. In most biological materials of animal origin, contamination by nickel, cobalt, or bismuth is of an order of magnitude that will not produce serious error since their color intensities are not over 5 per cent of that of copper. When bismuth interferes, carry out one determination with the usual technic. The result is copper and bismuth. To another sample before development of color add 5 ml. of 0.66 per cent potassium cyanide solution. Then develop the color which will be of bismuth alone. The difference between the two results represents copper. When cobalt and nickel interfere add 1 ml. of 0.5 per cent dimethylglyoxime solution to the sample before adding ammonia.[80]

[76] Thomas Callan and J. A. Russell Henderson, *Analyst* 54, 650-3 (1929); Stefan Ansbacher, Roe E. Remington and F. Barlow Culp, *Ind. Eng. Chem., Anal. Ed.* 3, 314-20 (1931); William D. McFarlane, *Biochem J.* 26, 1022-33 (1932); Sidney L. Tompsett, *Biochem. J.* 28, 1544-9 (1934); L. W. Conn, A. H. Johnson, H. A. Trebler and V. Karpenko, *Ind. Eng. Chem., Anal. Ed.* 7, 15-23 (1935); O. B. Winter, *J. Assoc. Official Agr. Chem.* 19, 359-65 (1936); Irwin Stone, *Ind. Eng. Chem., Anal. Ed.* 14, 479-81 (1942); D. F. Phillips and L. L. Edwards, *Metal Ind.* (London) 66, 409-10 (1945).

[77] M. Picotti and G. Baldassi, *Mikrochemie ver. Mikrochim. Acta* 30, 77 (1942).

[78] F. Grendel, *Pharm. Weekblad* 67, 913-21, 1345-51 (1930); W. Williams, *J. Dairy Research* 3, 93-100 (1931); L. A. Haddock and N. Evers, *Analyst* 57, 495-9 (1932).

[79] David L. Drabkin, *J. Assoc. Official Agr. Chem.* 21, 203-4 (1938); *ibid.* 22, 320-33 (1939); *ibid.* 23, 301-2 (1940); C. A. Greenleaf, *ibid.* 24, 337-48 (1941); H. W. Rusk, *ibid.* 25, 980-7 (1942).

[80] Lillian I. Butler and H. O. Allen, *ibid.* 25, 567-73 (1942).

Centrifuge to throw out nickel dimethylglyoxime and extract the color for determiuation.

Interference hy iron, uranium, and tricalcium phosphate in hiological samples when the color is to be extracted with organic solvent is prevented by addition of citric acid at pH8.5.[81] Alternatively, the iron is reduced to the ferrous state and fixed with α,α'-dipyridyl, the copper color developed later heing extracted with organic solvent. Lead[82] up to 8 mg. in the usual sample and zinc up to 16 mg. do not interfere. Mercury produces a very insoluhle compound with the reagent, hut this is overcome by use of excess reagent. More than 1 mg. of stannous compound causes the layer of extract to he cloudy, hut this can he centrifuged or filtered without effect on the color. Up to 8 mg. of stannic ion does not interfere. Large amounts of cadmium produce a white precipitate, insoluble in carhon tetrachloride, removable hy centrifuging or filtering. Arsenic and antimony do not give precipitates or colors in amounts up to 8 mg. Bismuth gives, in the ammoniacal solution, a yellowish green precipitate which dissolves in the carbon tetrachloride to a pale yellow instead of the golden brown of the copper salt. More than a trace of manganese gives a pink color in the solvent layer. This fades overnight in a stoppered tube in a warm place. Addition of 5 ml. of 4 per cent tetrasodium pyrophosphate after the solution is alkaline with ammonium hydroxide prevents this interference. Any chromium must be in the chromic form. Quantitative extraction of the copper compound is not affected adversely over the pH range of 5.7-8.5 provided large amounts of phosphate are not present. Above pH 8.5 there is a slight but continued reduction in color. Addition of electrolytes such as ammonium chloride, sulfate or citrate promotes the extraction with organic solvent.[83]

The method is, hy proper manipulation, applicahle in aqueous dispersion in the presence of iron, aluminum, lead, zinc, barium, and small amounts of cadmium. One technic is to buffer with ammonium citrate, read the color due to iron, and apply as a hlank.[84] Another is to sequester the iron with pyrophosphate.

The color is stahle for at least 1 hour. Cloudiness eventually develops due to oxidation of the reagent hut it will keep for several weeks in an amber hottle. Photoelectric methods are applicahle either with the color in aqueous solution or in organic solvent. The maximum ahsorp-

[81] E. J. Coulson, *ibid.* 19, 219-28 (1936); 20, 178 91 (1937); W. E. Parker and F. P. Griffin, *Can. J. Research* 17B, 66-70 (1939); F. W. Haywood, *Analyst* 68, 206-11 (1943).

[82] E. J. Coulson, *J. Assoc. Official Agr. Chem.* 20, 178-91 (1937).

[83] C. A. Greenleaf, *ibid.* 25, 385-92 (1942).

[84] T. P. Hoar, *Analyst* 62, 657-61 (1937).

tion is at 440 mμ but visual acuity is none too good in that region. It is best to use a green filter in a region where absorption is fair. With either white light or the mercury arc, 430-440 mμ is a desirable range.[85] A combination of Corning 511 with 038 Novial A to cut off the ultraviolet is satisfactory. Alternatives are Wrattan 29F, 62 and 75, each with Corning 430 at 0.95 mm. thickness.[86] Lovibond glasses standardized for this method are available.

Results with a standard deviation of less than 2 per cent have been obtained with fruit residues, using either acid digestion or stripping with nitric acid containing ammonium nitrate. Sulfuric acid used in ashing is frequently the source of a high blank. The method has been used to detect 0.005-0.05 mg. of copper per liter of water or copper in aluminum which is more than 99.99 per cent pure. By microphotometric manipulation determination of 0.003 mg. is possible.[87] Beer's law applies to the sysytem in carbon tetrachloride up to at least 2.5 ppm., and to 5 ppm. with a green filter. Solutions in organic solvent do not fade for some hours.

Procedure. *Absorption without Extraction.*[88] Take an aliquot of the sample solution which will contain 0.05-0.15 mg. of copper. Dilute to 25-40 ml. Neutralize this with concentrated ammonium hydroxide and add about 10 ml. in excess. If not already removed removed, heat the solution to boiling for a few minutes to coagulate iron and aluminum hydroxides, and filter. Wash the filter and discard. At this stage, if nickel is present to an interfering extent, add 5 drops of a 1 per cent solution of dimethylglyoxime in concentrated ammonium hydroxide. Let stand for 5 minutes and filter off the precipitate.

Transfer the cooled solution to a 100-ml. volumetric flask and add 25 ml. of concentrated ammonium hydroxide. Dilute to about 98 ml. remove a portion and use as the blank for adjustment of the photoelectric colorimeter at 100 per cent transmission with a 430 mμ filter. Return this to the flask, add 1 ml. of a 1 per cent solution of sodium diethyldithiocarbamate, and dilute to volume. With instruments using two photocells it is necessary to hold out enough solution for the comparison. Read the transmittance of the developed sample. Duplicates will usually agree within 0.003 mg.

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[85] B. Eisler, K. G. Rosdahl and H. Theorell, *Biochem. Z.* 285, 76-7 (1936); H. A. Schmidt, *ibid.* 302, 256-61 (1939); L. Braun and L. Scheffer, *ibid.* 304, 397-403 (1940).

[86] David L. Drabkin, *J. Assoc. Official Agr. Chem.* 22, 320-33 (1939).

[87] Alfred Eden and Henry H. Green, *Biochem. J.* 34, 1202-8 (1940); *cf.* Folke Nydahl, *Z. anal. Chem.* 116, 313-28 (1939).

[88] D. E. H. Frear, *Ind. Eng. Chem., Anal. Ed.* 11, 494-5 (1939).

Absorption with Extraction.[89] Transfer the aliquot to a 50-ml. tube. Dilute to approximately 20 ml. Add 4 ml. of 4 per cent tetrasodium pyrophosphate solution and mix. Add 5 ml. of saturated sodium citrate solution. Then add 1:4 ammonium hydroxide until alkaline to litmus. If nickel is present in interfering amounts add 5 drops of a 1 per cent solution of dimethylglyoxime in concentrated ammonium hydroxide. Let stand for 5 minutes and filter off the precipitate. Add 0.5 ml. of 0.5 per cent solution of the reagent and mix. Add 10 ml. of isoamyl alcohol, isoamyl acetate, bromobenzene, or carbon tetrachloride and shake vigorously for 0.5 minute. Pipet about 8 ml. of the organic solvent and centrifuge for 5-10 minutes to remove cloudiness. Alternatively dry with sodium sulfate. Read the transmittance and compare with a predetermined curve for which suitable samples will be 0.01-0.05 mg. of copper. If manganese is present let the solution stand for 20 minutes before reading.

Alternative Extraction Method.[90] Transfer an aliquot, containing not over 0.05 mg. of copper, to a 60-ml. separatory funnel. Add 2 ml. of 40 per cent ammonium citrate solution, adjusted to pH 8.5 with ammonium hydroxide. The ammonium ion is present to deionize iron[91] and prevent rapid fading of the developed color. Add 10 ml. of 0.1 per cent diethyldithiocarbamate solution and 10 ml. of isoamyl acetate. Shake for 5 minutes and let the acetate layer separate. Remove the nonaqueous layer and centrifuge for 5 minutes to separate water droplets. Read the transmittance.

Successive extractions with four 2.5-ml. portions of carbon tetrachloride, or more if needed to complete the extraction or reduce the color, are also used.[92] Lovibond yellow glasses are a suitable form of permanent comparison standard.

Duplication.[93] Add a small piece of litmus paper as indicator to an aliquot containing 0.02-0.1 mg. of copper. If lead is known to be present add about 5 ml of 1 per cent ferric chloride solution and mix. Add concentrated ammonium hydroxide until just alkaline. Add 2 ml. excess

[89] P. le Roux van Niekerk, *Onderstepoort. J. Vet. Sci. Animal Ind.* 9, 623-8 (1937).

[90] R. Q. Parks, S. L. Hood, Charles Hurwitz and G. H. Ellis, *Ind. Eng. Chem., Anal. Ed.* 15, 527-33 (1943).

[91] Alfred Eden and Henry H. Green, *Biochem. J.* 34, 1202-8 (1940).

[92] L. A. Haddock and Norman Evers, *Analyst* 57, 495-9 (1932).

[93] G. F. Palfrey, R. H. Hobart, A. F. Benning, and I. W. Dobratz, *Ind. Eng. Chem., Anal. Ed.* 12, 94-6 (1940).

of concentrated ammonium hydroxide, heat to boiling, and digest on the steam bath until aluminum and iron are coagulated. For complete precipitation of aluminum this requires at least 1 hour. Filter into a 100-ml. tube and wash the paper well with hot water. Dilute the cooled solution in the tube to about 50 ml. Add 1 ml. of 5 per cent gum arabic solution and mix. Add 10 ml. of concentrated nmmonium hydroxide and mix. Add 10 ml. of 0.1 per cent solution of the reagent and mix. If turbidity is present and is due to zinc or cadmium, add 10 ml. more of concentrated ammonium hydroxide, and if necessary 10 ml. more. Finally dilute to volume. If still turbid use the special technic at the end of this procedure. If the color is too dark, repeat with another aliquot; do not dilute the developed sample. If turbidity is present and cannot be removed by one of the precautions cited, the method is not applicable.

In another similar tube take all of the reagents present in the aliquot of sample, treat in the same way as the sample and dilute to about 90 ml. Add a suitable standard copper solution, usually containing 0.01 mg. of copper per ml., until the color of the sample is matched, and complete as usual by matching both color nnd volume.

When zinc or cadmium have to be removed, dilute the aliquot to about 25 ml. and neutralize to litmus with concentrated ammonium hydroxide. Add 5 ml. of concentrated hydrochloric acid, mix, and pass hydrogen sulfide through for about 10 minutes. Filter and, without washing, transfer the paper to the original beaker. Discard the filtrate. Add 10 ml. of 1:3 sulfuric acid and boil for n few minutes. Add 25 ml. of water, filter, and wash the filter well with hot water. Use this filtrate as sample starting at "Add concentrated ammonium hydroxide until just alkaline."

Balancing. Develop a comparable standard at the same time as the sample, since the color darkens somewhat with time.

Copper by Dithizone

This reagent is applicable to many metals as discussed in general under lead.[94] In its application to copper,[95] the interfering metals are

[94] For a more detailed discussion of this reagent and precautions necessary for its use refer to page 3.

[95] Hellmut Fischer and Grete Leopoldi, *Wiss. Veroffentlich. Siemens-Konzern* 12, 44-52 (1933); Angew. Chem. 47, 90-2 (1934); Hellmut Fischer, *ibid.* 50, 919 32 (1937); R. M. Mehurin, *J. Assoc. Official Agr. Chem.* 18, 192-4 (1935); Erich Stolze, *Bodenk. u Pflanzenernähr.* 1, 115-32 (1936); H. A. Liebhafsky and E. H. Winslow, *J. Am Chem. Soc.* 59, 1966-71 (1937); E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 9, 464-9 (1937); O. Braadlie and H. Bergen, *Tids. Kjemi. Bergvesen. Met.* 2, 88-9 (1942); C. A. Greenleaf, *J. Assoc. Official Agr. Chem.* 30, 144-52 (1947).

normally separated. The keto form in carbon tetrachloride gives a violet color. Copper is extractable with dithizone nearly down to pH 1 but is most readily extracted above pH 5.[96] This is necessarily tempered by the necessity of avoiding interference. When dithizone extraction of copper in the presence of lead is relied on for separation, it is well to work at pH 0.5-1.0 and rely on multiple extractions.[97] Over 50 mg. of ferric iron will interfere by oxidation of the reagent, but can be removed by ether extraction. The original method, applicable with accuracy only to 0.03-0.05 mg., has been modified to be applicable with accuracy to 0 0003 mg. by extraction and concentration into smaller volumes. In some of the modifications, great accuracy in technique is necessary. Sodium pyrophosphate is sometimes substituted for the ammonium hydroxide of the original method because the latter bleaches very minute amounts of the copper-dithizone complex. The pyrophosphate also retards the interference of tin. For accuracy, filtration and contact with flocculent precipitates must be avoided.

The intensity of color developed in the organic solvent corresponds to Beer's law up to 1 ppm., and it is only necessary to insure the absence of interfering metals in the final extract to obtain accuracy. A solution of dithizone in chloroform or carbon tetrachloride, extracts from solutions of pH below 3, only gold, platinum, palladium, silver, mercury, bismuth, stannous tin, and copper. At this pH other metals do not react.[98] Impure carbon tetrachloride can lead to low results due to incomplete extraction.

Substantial amounts of zinc, cadmium, and bismuth do not ordinarily affect the quantitative extraction under any conditions, and silver, mercury, and stannous tin do not if dithizone is in excess. By oxidation of tin to stannic ion, the possible interference is removed. Similarly, possible interference by bismuth can be eliminated by extraction of the carbon tetrachloride solution with acidified potassium iodide solution.[99] Interference by iron can be eliminated by introduction of hydrazine sulfate which will reduce the iron to the ferrous state.[100] Excess dithizone is adequately removed by washing with 1:200 ammonium hydroxide, but the mixed color method is preferred. The monocolor method can be

[96] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66 72 (1939).
[97] P. M. Heertjes, *Chem. Weekblad* 42, 91 5 (1946).
[98] Hellmut Fischer, *Angew. Chem.* 47, 685 92 (1934).
[99] C. A. Greenleaf, *J. Assoc. Official Agr. Chem.* 25, 385 92 (1942).
[100] G. Schwarz, O. Fischer and H. Stolz, *Milchwirtschaft. Forsch.* 17, 314-25 (1936); G. Schwarz and O. Fischer, *ibid.* 18, 196 204 (1936).

used,[101] but with lessened accuracy. The ferric ion can also be removed by titration with 1 per cent titanium trichloride solution to disappearance of the yellow color.[102]

The rate of reaction of copper with dithizone is slow, so that at least 6 minutes' shaking at 275 cycles per minute is required for reasonably complete removal. This is indicative of the desirability of using a mechanical shaker for extraction. One variation [103] of steps comprises (1) extraction at about pH 3.1 in the presence of iodide ion with a large excess of dithizone in chloroform, (2) shaking the dithizone extract with iodide to remove traces of silver, mercury, bismuth, zinc or cadmium, (3) decomposition of the dithizone with bromine water, (4) extraction of the copper with 0.01 *N* hydrochloric acid, (5) buffering at pH 2.9, and (6) titrametric extraction.

The usual sample for analysis determines 0.001-0.05 mg. of copper with accuracy better than 1 per cent. The method has also been used titrametrically.[104]

Procedure. *Mixed-color Method.* Transfer a suitable sample to an evaporating dish and add 10 ml. of 1:10 sulfuric acid. Heat on a sand bath to sulfur trioxide fumes, let cool, and take up with 20 ml. of water. Transfer to a 50-ml. volumetric flask and dilute to volume with water. The final solution should contain not over 2 per cent of sulfuric acid.

Shake the sample solution for 2 minutes with a small portion of the reagent containing not over 6 mg. of dithizone per 100 ml. of carbon tetrachloride. Repeat this with successive portions until, after shaking, the reagent shows the greenish color of free dithizone instead of the red-violet of copper dithizonate. Dilute the combined extracts with carbon tetrachloride to 20 ml. Shake the extract with 5-10 ml. of 0.7 per cent sodium thiosulfate solution to reduce ferric iron. Wash the carbon tetrachloride layer with two 5-ml. portions of water. Finally filter or centrifuge the carbon tetrachloride solution to remove suspended droplets of water and read the absorption with a 510 or 625 mμ filter, or compare with a standard or series of standards developed in the same way at the same time.

[101] J. Schwaibold, B. Bleyer and G. Nagel, *Biochem. Z.* 297, 324-31 (1938).

[102] K. Scharrer and H. Kühn, *Bodenkunde u. Pflanzenernahr. Z.* 21-2, 344-64 (1940).

[103] C. A. Greenleaf, *J. Assoc. Official Agr. Chem.* 30, 144-52 (1947).

[104] A. G. Assaf and W. C. Hollibaugh, *Ind. Eng. Chem., Anal. Ed.* 12, 695-7 (1940).

in Figure 7. From this read the values to prepare the nomograph, Figure 8.

For the determination take an aliquot of sample falling within the range of the nomograph and adjust the volume and acidity so that it is

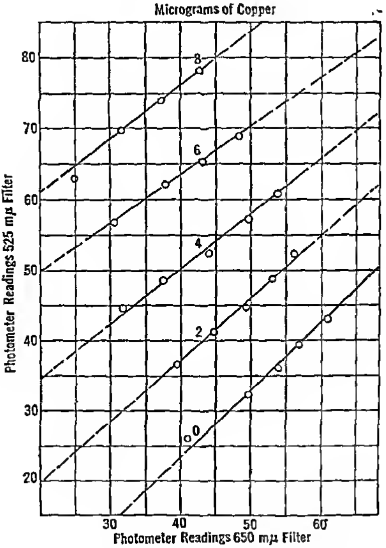

FIG. 7

Photometric Relationship at 525 and 650 mμ

approximately 0.1 N with hydrochloric acid. Add 10 ml. of the dithizone solution of the concentration used in preparing the nomograph. Shake for 5 minutes and filter the carbon tetrachloride layer through cotton into the cell to read. Measure the transmittance at 525 mμ and 650 mμ.

To translate, select the line of the nomograph corresponding to the 650 mμ value. Determine its intersection with the 525 mμ axis. Read the copper concentration from the abscissae.

Monocolor Method.[106] Prepare a buffer for pH 2.3. For this dissolve

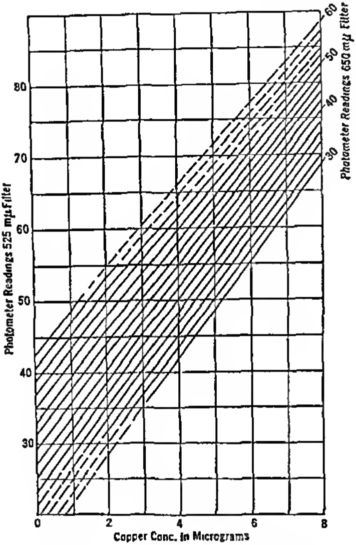

FIG. 8

Nomograph for the Dithizone copper Dithizonate System

38 grams of citric acid and 21 grams of disodium hydrogen phosphate dodecahydrate, $Na_2HPO_4 \cdot 12H_2O$, of equal quality, in water and remove interfering metals (page 3). Dilute the aqueous layer to 250 ml. as a stock solution. Add 2 ml. of this to water, dilute to 25 ml., and add 2 drops of 0.02 per cent aqueous solution of cresol red.

[106] G. H. Bendix and Doris Grabenstetter, *ibid.* 15, 649-52 (1943).

Pipet an aliquot of sample solution containing 0.002-0.010 mg. of copper into a 150-ml. separatory funnel. Add sufficient 1:10 sulfuric acid to dilute to 25 ml. Add 2 drops of a 0.02 per cent aqueous solution of cresol red and add concentrated ammonium hydroxide until the color approximates that of the buffer. Add 2 ml. of the stock buffer solution and mix. Then add exactly 10 ml. of a solution containing 15 mg. of pure dithizone per liter in carbon tetrachloride. Shake mechanically for 10 minutes. Let the carbon tetrachloride separate and draw it off into another separatory funnel.

Dissolve 10 grams of potassium iodide in 450 ml. of water and add 5 ml. of 1:360 hydrochloric acid. Add 2.5 per cent sodium thiosulfate dropwise until the color of free iodine is just destroyed. Shake with 10 ml. portions of the dithizone solution until no further discoloration of the dithizone occurs. Wash the acidified iodide solution with 10 ml. of carbon tetrachloride. Discard all of the washings and diluta the aqueous layer to 500 ml. In use, check this at least once a day for free iodina and, if present, remove it with sodium thiosulfate solution.

Add 10 ml. of this acidified iodide solution to the carbon tetrachloride extract of the sample. Shake for 2 minutes and let separate. This extracts any silver, mercury, or bismuth present. Transfer the carbon tetrachloride layer to another separatory funnel and shake with 25 ml. of 1:200 ammonium hydroxide solution. This removes excess reagent. Use the carbon tetrachloride solution for determination of the color, preferably by transmittance at 520 mμ. Comparison against a natural standard by balancing is satisfactory as the system follows Beer's law in the region concerned. The colors can also be read against Lovibond glasses.[107] A further modification is to carry out the extraction in a Mojonnier extraction flask, avoiding transfers from separatory funnel to separatory funnel.[108]

Copper by Ammonium Hydroxide and Amines

A solution of a cupric salt, on adding ammonium hydroxide, gives an intense blue color of the cupric-ammonia complex, which is proportional in intensity to the amount of copper present. The method is very old [109] and has been applied to samples of nearly every type. Spectro-

[107] A. J. van Duuren, *Chem. Weekblad* **37**, 269 (1940).

[108] S. L. Morrison and Harriet L. Paige, *Ind. Eng. Chem., Anal. Ed.* **18**, 211-13 (1946).

[109] Heine, *Bergwerksfreund* **1**, 33 (1830).

photometric studies[110] have added greatly to the knowledge of its applicability.

Limits on permissible concentrations of anions which form complexes without change in hue are citrate 100 ppm., molybdate 200 ppm., silicate 250 ppm., tartrate 100 ppm., thiosulfate 350 ppm. Many others at 500 ppm. cause a negligible effect, defined as less than 2 per cent variation. But pyrophosphate and salicylates alter the hue, dichromates interfere by their own color, and chlorostannate, chlorostannite, tungstate, and vanadate cause turbidity by hydrolysis or reaction with copper.

Nickel and cobalt interfere by forming colored complexes. The colorless complexes with silver, cadmium, and zinc ions do not interfere. Interference by barium, lead, and strontium in the presence of sulfate is due to precipitation. Precipitation of hydroxides or basic salts of aluminum, antimonous, beryllium, bismuth, chromic, ferric, ferrous, magnesium, manganous, mercuric, mercurous, thorium, uranyl, and zirconium ions interferes. Fuming with sulfuric acid followed by taking up with ammonium hydroxide and filtration eliminates these.

The presence of ammonium sulfate has negligible effect but a positive error of 2 per cent arises from 200 ppm. of ammonium chloride. Therefore it appears that chloride interferes and it is desirable to vaporize chlorides, even though the error apparently becomes constant at +3.3 per cent. In dilution methods the menstruum used must have the same concentration of ammonium hydroxide as the sample and standard.

The citric-acid complex results only in the solution becoming greenish.[111] This can be corrected by a yellow or red filter. The citric acid complexes are not affected by air in 24 hours but aluminum must not be present if manganese is so fixed. An alternative to fixation as the complex is precipitation as the hydroxide.[112]

When studied spectrophotometrically in 3 *N* ammonium hydroxide, the maximum absorption is found at 620 mμ. Over the range 40-600 ppm. the logarithm of the transmittances against concentration gives a straight line showing conformity to Beer's law over that range.[113] The intensity of blue color varies according to the concentration of ammo-

[110] J. Pfanhauser and J. Jacewiczówna, *Przemysl Chem.* 21, 150-2 (1937); John H. Yoe and Charles J. Barton, *Ind. Eng. Chem., Anal Ed* 12, 456-9 (1940); J. P. Mehlig, *ibid.* 13, 533-5 (1941); *ibid.* 14, 903 (1942); Oscar I. Milner, *ibid.* 18, 94-6 (1946).

[111] Rea Schubert, *Angew. Chem.* 54, 87-9 (1941).

[112] Erich Bischof and Georg Geuer, *Metal u. Erz* 41, 57-63 (1944).

[113] Ralph H. Müller, *Ind Eng. Chem., Anal Ed.* 7, 223-6 (1935); John H. Yoe and Thomas B. Crumpler, *ibid.* 7, 281-4 (1935); John H. Yoe and Charles J. Barton, *ibid.* 12, 456-9 (1940).

nium hydroxide. The wave length of maximum absorption also increases with ammonium hydroxide concentration. The color appears to be stable indefinitely so that permanent standards need only be kept tightly stoppered and in diffuse light, provided Pyrex glass is used to minimize reaction with the glass. So a desirable concentration of ammonium hydroxide in the final solution is about 3 N or about 1:4 but this need not be accurate provided the amount in sample and standard is similar.

By suitable choice of aliquots, the method is accurate for samples containing as much as 10 per cent of copper, with accuracy to 0.1 per cent.

To obviate the strong odor and loss of volatile reagent, amines have been studied and details for use of triethanolamine are given. It is to be expected that mono- and diethanolamine, aminomethylpropanol, and other amines would be equally satisfactory. The study was with a photoelectric spectrophotometer using only a 10 mμ band centered at 625 mμ. Above 3.5 per cent of triethanolamine the intensity of 100 ppm. of copper remains substantially constant. Having the triethanolamine present as a salt accentuates the color. Potassium and sodium salts decrease the color. The photometric method has been applied to continuous recording for control purposes.[114]

The copper complex with triethanolamine deviates somewhat from Beer's law below about 80 ppm. of copper. The reaction has slightly greater sensitivity at low concentrations of copper. When excess of ethylene diamine is used the system follows Beer's law.[115] The work was done at 1.2 N and readings were with a 546 mμ filter.

Another amine reagent is 1,2-diaminoanthraquinone sulfonic acid.[116] By this reagent alone, if copper is less than 2 mg. per liter, the error is about 10 per cent, but 4-12 mg. are determined within about 1.8 per cent. By addition of potassium hydroxide the method is about 100 times as sensitive as that with ammonium hydroxide, determining 0.04-0.2 mg. of copper within 1.2 per cent.

When tetraethylenepentamine is the reagent, the blue color is independent of excess amine and the system conforms to Beer's law.[117] It is twice as sensitive as triethanolamine and 3.5 times as sensitive as ammonium hydroxide. The color is stable for at least 48 hours.

Procedure. *Interfering Metals Absent.* This assumes the absence of

[114] Earl H. Brown and James E. Cline, *ibid.* **17**, 284-5 (1945).
[115] S. E. Q. Ashley, *ibid.* **11**, 72-9 (1939).
[116] J. Sebor, *Chem. Listy* **31**, 419-20 (1937).
[117] Thomas B. Crumpler, *Anal. Chem.* **19**, 325-6 (1947).

hydroxides or basic salts insoluble in ammonium hydroxide. To 25 ml. of sample add 1:1 ammonium hydroxide with a graduated pipet until the copper reaches incipient precipitation, noting the approximate volume used. Add one-third of the final volume in terms of 1:1 ammonium hydroxide, thus providing for the final solution to be 3 N with ammonium hydroxide.

As standard add to a known volume of copper standard the amount of any reagents other than ammonium hydroxide and acid used in preparing the sample. Now add the amount of 1:1 ammonium hydroxide required to neutralize the sample. Titrate this back from a pipet with a 1:1 dilution of the acid which was present in the sample. This will nearly always be sulfuric acid. The end point is reached when the cupric hydroxide has precipitated and redissolved. If the titration is substantial it will be desirable to cool the solution. Now add the same volume of 1:1 ammonium hydroxide to the standard as to the sample to develop the color and dilute to the same volume as the sample. Compare sample and standard by balancing. The duplication method can be applied, using a solution of the same salts as in the sample with sufficient ammonium hydroxide to make the final solution 3 N, which is about 1:4 dilution of concentrated ammonium hydroxide.

Alternatively determine the transmittance. For this prepare a blank from one portion of the sample solution by adding sufficient 6.5 per cent potassium cyanide dropwise to the alkaline sample in the cell to just remove the color. For two-cell instruments compare the developed solution in a 2-cm. cell against this blank at 570, 580, and 590 mμ. For single cell instruments set the reading at 100 with the blank and then substitute the developed sample. It is best to calculate at the three wave lengths and average as the analysis, although a reading at a single wave length can be used with somewhat lessened accuracy.

Interfering Metals Present. If the sample may still contain metastannic acid or a hydroxide insoluble in ammonium hydroxide, use this procedure. To 25 ml. of sample solution add 1:1 ammonium hydroxide with a graduated pipet until the copper reaches incipient precipitation. Other metals may precipitate. Now add about one-third of the final volume in terms of 1:1 ammonium hydroxide, and filter into a 250-ml. volumetric flask. Wash the residue on the paper with 1:4 ammonium hydroxide until the washings are colorless. Wash the residue from the paper back into the casserole without damaging the paper. Dissolve any soluble hydroxides by dropwise addition of concentrated sulfuric acid until it is in excess. Reprecipitate by addition of 1:1 ammonium hydrox-

ide until at the point of incipient precipitation and add torn bits of filter paper to hasten filtration. Add an amount of 1:1 ammonium hydroxide equal to approximately one-third of the volume. Filter into the flask and wash the precipitate and paper with 1:4 ammonium hydroxide until nearly to the mark. Discard the filter and dilute to volume with 1:4 ammonium hydroxide at a known temperature. Mix well. The double precipitation provided is more accurate and convenient than displacement by aluminum. The copper left in the residue after the second precipitation never exceeds 0.04 per cent. Compare as described when interfering metals are absent.

Development with Triethanolamine. Follow the procedures already outlined for ammonium hydroxide but substitute triethanolamine for ammonia. If desired, for economy, the amount used may be cut back to 3 per cent of the free amine.

Copper in Concentrated Halogen Acid

If no other metals whose halides are also deeply colored are present, the concentration of a copper solution in concentrated halogen acid may be determined by the intensity of the color produced.[118] The color of the chloride is yellow, that of the bromide violet. In concentrated hydrobromic acid, ferric iron forms an amber complex and both iron and copper can be determined in the same solution by photometric methods.[119] The spectra overlap so that a correction must be applied to each determination for the other. There is no interference by elements up to the limits cited: aluminum 12 per cent, zinc 5 per cent, manganese 2 per cent, calcium 1 per cent, lead 0.5 per cent, silicon 0.8 per cent, nickel 0.05 per cent. There may be interference by molybdenum, vanadium, chromium, cobalt, gold, the platinum metals, and some rare earths.

The hydrobromic acid must be water-white and usually needs to be redistilled in glass. Then bromine goes over in the first fraction and metals remain in the residue. It is best stored in a bottle which has been soaked with hydrochloric acid and painted black to protect the contents from light.

In determination as the chloride, when iron must be absent, the

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[118] C. Huttner, *Z. anorg. Chem.* 86, 351 (1914); Georges Deniges and E. Simonot, *Bull. soc. pharm. Bordeaux* 54, 337-40 (1915).

[119] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 323-5. American Society for Testing Materials, Philadelphia, Pa. (1946).

intensity of color reaches its maximum at a hydrochloric-acid concentration of 28 per cent, and after that remains constant. Free chlorine does not interfere but bromine, nitric acid, or nitrogen oxides do. Photoelectrically, 0.008 mg. in 1 ml. of hydrochloric acid is determinable to 1.6 per cent, 0.002 mg. similarly in hydrobromic acid. It conforms to Beer's law only over a short range. Cobalt or nickel will have very little color in the concentrated acid solution unless the amount is large. Manganese does not interfere. Minute amounts of organic matter may produce a yellow color and if present must be burned out before dissolving in acid. The yellow coloration due to iron is to that of copper as 9:5.

A modification [120] is to add potassium bromide in 1:2 sulfuric acid to the aqueous solution of sample to which concentrated sulfuric acid has been added to raise the acidity similarly.

Procedure. *As the Bromide.* Take an aliquot of sample, usually 10 ml., which has been dissolved in hydrobromic acid and from which other acids are absent. It is desirable that this sample contain 0 02-0.25 mg. of copper and if iron is to be determined it should fall in the range of 0.006-0.12 mg. Many preparations of samples are applicable by use of hydrobromic acid instead of hydrochloric acid. To the aliquot add sufficient saturated bromine water to give a yellow color and evaporate to less than 1 ml. of liquid but not to absolute dryness. Add 5 ml. of 48 per cent hydrobromic acid and if necessary warm to dissolve the bromides. If insoluble matter is present, filter through a fritted glass crucible and wash with 1-2 ml. of 48 per cent hydrobromic acid.

Transfer the clear liquid to a 10-ml. volumetric flask and dilute to volume with 48 per cent hydrobromic acid. Mix, read the transmittance at 600 mμ and 460 mμ and compare with calibration curves. Read within 1 hour as the color due to copper darkens on standing. When the transmittance is below 20 per cent at 460 mμ or 50 per cent at 600 mμ, dilute the sample with more 48 per cent hydrobromic acid, or read in a thinner cell. If diluted with water the color fades.

The reading of two colors has been discussed in Vol. I, page 18, and in this chapter at page 115. As a first approximation assume that the reading at 600 mμ measures copper and that at 460 mμ measures iron. If the values do not differ greatly or if the iron is lower, assume the

[120] Georges Denigès and E. Simonot, *Bull. soc. pharm. Bordeaux* 54, 337-40 (1915).

copper figure to be correct and use it to correct the iron value. If the iron figure is markedly higher use it to correct the copper value. If the iron is below 0.02 mg. no correction need be applied to the copper value. The equation for correction is as follows:

$$T_{Fe} \times T_{Cu} = 100 \times T_{obs}$$

where

T_{Fe} = percentage transmittance in the presence of iron alone
T_{Cu} = percentage transmittance in the presence of copper alone
T_{obs} = percentage transmittance observed for the mixture

Necessarily this involves transmittance curves for each at each wave length and calculations are limited to values at one wave length.

Thus assume the copper value to have been read on the 600 mμ curve. Look up that value for copper on the 460 mμ curve and substitute in the above equation. Also substitute T_{obs} and solve for T_{Fe}. Finally read that value from the 460 mμ curve for iron to get the corrected iron concentration. Similarly use that corrected iron concentration read from the iron curve at 600 mμ to get the corrected copper value.

As the Chloride. Select an aliquot of sample containing about 0.01 gram of copper. If the aliquot contains interfering metals make distinctly acid with hydrochloric or nitric acid, add 0.5 gram of potassium chlorate, dilute with water, and precipitate copper as the sulfide by washed hydrogen sulfide. Filter the copper sulfide and ignite. Dissolve the cupric oxide in 10 ml. of 1:1 hydrochloric acid and dilute to 100 ml. with concentrated hydrochloric acid.

If the aliquot is free from interfering metals, evaporate to dryness, add more hydrochloric acid, and repeat the process twice. Dissolve the residue in a small volume of 1:1 hydrochloric acid and then add concentrated hydrochloric acid to make a volume of 100 ml.

In either case compare by transmittance, by balancing, or by dilution with concentrated hydrochloric acid.

Copper by Pyridine and Thiocyanate

When copper is treated in aqueous solution with pyridine and an alkali thiocyanate, an insoluble compound, $[Cu(C_5H_5N)_2](CNS)_2$, is precipitated. This compound is soluble in chloroform or bromobenzene to give a yellow-green color, and when so dissolved is suitable for colori-

metric estimation.[121] As usually carried out the organic solvent is present with the other reagents and the precipitate is never separated. Because of the presence of thiocyanate, iron must be absent or tied up as a complex. For this purpose citric acid is sometimes added. Interference of iron is also prevented by adding 1 ml. of 10 per cent tartaric acid to the aliquot of sample and allowing the acidified solution to stand for a few minutes before treatment with the reagents. Manganese does not interfere. Ferrous and mercurous salts must be oxidized, and nickel, cobalt, and silver must be absent.

Accuracy within 4 per cent is readily obtained and with care can be considerably better. Satisfactory results have been obtained with less than 0.1 mg. of copper in the presence of 40 mg. of iron. With sample and standard similar in color 0.01-0.05 mg. may be estimated. Some large errors occurring in milk ashed by the dry method or in recovery of 0.1 mg. of copper from 10-20 grams of liver[122] are probably due to the methods of preparation of the samples. If hydrogen peroxide has been used as an oxidizing agent in preparation of the sample, it must be completely removed, as by boiling for 10 minutes.

An equivalent reaction with *o*-tolidine (3,3'-dimethylbenzidine) and thiocyanate forms tolidine blue.[123] As compared against a color standard the result is within 2.5 per cent in determination of 0.005-0.2 mg. of copper. Iron interference is obviated by fluoride, the colloidal color stabilized with gelatin and acid buffered with sodium acetate. Development of color takes place in 10 minutes.

Procedure. Transfer a suitable aliquot to a 50-ml. tube and dilute to about 35 ml. In another tube take a suitable amount of standard containing 0.1 mg. of copper per ml., and dilute to about 35 ml. Make

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121 G. Spacu, *Bul. soc. stiinte Cluj* **1**, 352-5 (1922); *Analyst* **49**, 275 (1924); R. Biazzo, *Ann. chim. applicata* **16**, 96 (1926); R. Schonheimer and F. Oshima, *Z. physiol. Chem.* **180**, 249-52 (1929); Hans Kleinmann and Joachim Klinke, *Arch. path. Anat.* **275**, 422 (1930), Leslie J. Chalk, *Analyst* **55**, 187-91 (1930); Ch. Benoit, *Ann. chim. anal. chim. appl.* **12**, 66-9 (1930); H. T. Gebhardt and H. H. Sommer, *Ind. Eng. Chem., Anal. Ed.* **3**, 24-6 (1931); Jackson B. Hester, *Chemist-Analyst* **25**, 78-9, 83 (1936); Joseph Krenn, *Mikrochemie* **23**, 149-59 (1937); Roland A. Bosee and Paul Fehder, *J. Am Pharm. Assoc.* **29**, 141-2 (1940); S. S. Korol, *Pedology* (U.S.S.R.) 1940, No. 3, 126 32; L. A. Gulyaeva and E. S. Itkina, *J. Applied Chem.* (U.S.S.R.) **17**, 252 8 (1944).

122 Hugh J. Kearney and Edmund J. Virnsky, *Mendel Bull.* **9**, 71-4 (1937).

123 R. I. Lirtzman and L. M. Kul'berg, *Voprosui Pitaniya* **5**, No. 3, 45-50 (1936); L. M. Kul'berg, *ibid.* **8**, No. 5, 75-9 (1939); *Khim. Referat. Zhur.* 1940, No. 5, 82.

any additions of salt or acid to the standard to have it correspond approximately to the sample. If at this stage the sample and standard are acid, approximately neutralize with 1:4 ammonium hydroxide.

Add 3 ml. of glacial acetic acid, 3 ml. of 10 per cent ammonium thiocyanate solution, and about 2.5 ml. of pyridine to each. Mix well and add 5 ml. of chloroform to each. Shake well and let the chloroform separate. Remove the chloroform layers and compare by balancing, preferably by micro methods. Alternatively omit the standard and determine the transmittance.

Copper by *o*-Phenanthroline

When an ammoniacal solution of a cupric salt is treated with *o*-phenanthroline and reduced, a strong orange to brown color develops.[124] This is suitable for determination of copper[125] by a method closely analogous to that for iron. The ammonium hydroxide concentration must be carefully controlled and the color reagent must precede the reducing agent. Hydroxylamine is suitable as the latter. By having a water-soluble solvent present, the colored compound does not precipitate, hue does not change with copper concentration, and the system conforms to Beer's law. Extraction of the color has not been found feasible. There is no advantage of sensitivity or stability in the use of the 5-chloro, 5-bromo, 5-methyl, 5-nitro or 5-methyl-6-nitro derivatives of the reagent. Less sensitivity results with 2,2′-dipyridyl, and 2,2′,2″-terpyridyl does not react. As solvent, methyl Carbitol was found most satisfactory and its concentration is not critical. If examined photometrically at 435 $m\mu$, Beer's law is valid for at least 0.5-10 ppm. Corning 556 filter is suitable.

The error arising from 500 ppm. of most common ions with 5 ppm. of copper is less than 2 per cent. Precipitation occurs with aluminum, antimony, bismuth, cerium, chromium, cobalt, iron, lead, manganese, mercury, silver, titanium, thorium, uranium, zirconium, and more than 100 ppm. of beryllium. Cadmium, cobalt, and zinc form complexes with the reagent. The only anions found to interfere were cyanide, thiosulfate, and large amounts of dichromate and metavanadate. Iron must be removed by precipitation with ammonium hydroxide. When the concentration is high enough there is no advantage over the ammonium

[124] G. Tartarini, *Gazz. chim. ital.* **63**, 597-600 (1933).

[125] M. L. Moss and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 14, 931-3 (1942); *ibid.* **15**, 116-18 (1943).

hydroxide method. At low concentrations the method is applicable over 0.1 ppm. but is less sensitive than diethyldithiocarbamate.

Procedure. Take a suitable aliquot of sample solution to contain 0.01-0.5 mg. of copper. Dilute or concentrate to about 15 ml. If precipitable metals are present, add concentrated ammonium hydroxide until the solution is just alkaline to litmus. Filter into a flask and wash the filter with 1:4 ammonium hydroxide until the washings are colorless. Set the filtrate aside and transfer the precipitate to the original container with water. Dissolve this precipitate by dropwise addition of 1:1 sulfuric acid. Precipitate, filter, and wash as before using the same filter and receiver. Discard the paper, and concentrate the filtrate to about 15 ml.

Neutralize the solution to litmus by addition of 1:2 hydrochloric acid or, if the previous precipitation has not been required, in some cases with 1:1.5 ammonium hydroxide. Add the following in the order stated, mixing after each addition: 2 ml. of 1:1.5 ammonium hydroxide, 10 ml. of 0.1 per cent solution of *o*-phenanthroline, 1 ml. of 10 per cent hydroxylamine hydrochloride solution, 20 ml. of methyl Carbitol. Dilute with water to 50 ml. Read the transmittance or compare by balancing or dilution against a standard similarly treated. The color is stable for at least 24 hours.

Copper by Potassium Ferrocyanide

The amount of copper in dilute acetic acid solution may be estimated from the intensity of the reddish brown color produced by reaction with potassium ferrocyanide. The method is suitable for application when the color with ammonium hydroxide is too light a blue. Up to 2 ppm., the color is a turbidity rather than a precipitate.[126] If the solution is strongly acid the color turns to brown, if alkaline it fades.

Photometric methods are suitable [127] for determination of 0.8 ppm. in 1 ml. with an accuracy to 1.6 per cent.[128] On a larger scale it is accurate to 0.5 per cent.[129] The system follows Beer's law. The method is one of examination of a colloidal precipitate which begins to show

[126] Pierre Thomas, *Biochem. Z.* 293, 396-8 (1937); Robert Juza and Robert Langheim, *Angew. Chem.* 50, 255-9 (1937).

[127] S. I. Sinyakova, *J. Applied Chem.* (U.S.S.R.) 10, 2109-17 (1937).

[128] Ralph H. Muller and A. T. Burtsell, *Mikrochemie ver. Mikrochim. Acta* 28, 209-28 (1940).

[129] Harry Hahn, R. Juza and R. Langheim, *Z. anal. Chem.* 110, 270-5 (1937).

acid. Dissolve the sample and standards in 1 ml. of water and transfer each to a tube. To each add 1 ml. of this buffer solution. Add 1 ml. of a fresh 0.1 per cent cryogenine solution, dilute to 3.5 ml., and mix. Cover and place in an incubator or a water bath at $39.5 \pm 0.1°$ for 1 hour. Compare the sample by balancing with the standard nearest in color, using a microcolorimeter, or read the transmittance of the sample against a prepared curve. For routine photoelectric determination discard the results if the one standard does not fall on the curve.

Copper by Potassium Ethyl Xanthate

Small amounts of copper in solution react with potassium ethyl xanthate to produce a yellow color suitable for colorimetric estimation.[133] Small amounts of iron, lead, nickel, cobalt, zinc, or manganese do not interfere. Nickel gives a color very similar to that of copper if present in significant amounts.

As applied in 25-ml. volumes the error is 10-15 per cent on 0.005-0.018 mg. of copper and, at 0.001-0.002 mg., becomes as great as 50 per cent. This definitely establishes a lower limit of its application. Opalescence interferes if the solution is far from neutral. At 0.02 mg. per final 25 ml., pH 7.0-8.5 is best although turbidity develops with time. As a general condition, pH 7.0 with 0.005-0.020 mg. of copper per final 25 ml. is probably best. Comparison should then be within 15 minutes.

Procedure. Place 10 ml. of a 0.1 per cent solution of potassium ethyl xanthate in each of two Nessler tubes of suitable size. Add a suitable aliquot of the sample to one tube and dilute to 50 or 100 ml. The yellow color appears at once. In the other tube, dilute the potassium ethyl xanthate nearly to volume and add a standard copper sulfate solution containing 0.01 mg. of copper per ml. until the color is substantially duplicated. Complete as usual.

To confirm the correctness of this duplication it is desirable to prepare a series of 5 standards. One is to contain the amount of standard estimated by duplication. The others contain 0.05 and 0.10 ml. more and 0.05 and 0.10 ml. less of the standard than was estimated to be present by duplication. Determine the final estimate of the sample from

[133] G. C. Supplee and B. Bellis, *J. Dairy Sci.* 5, 455 (1922); L. H. Lampitt, E. B. Hughes, P. Bilham and C. H. F. Fuller, *Analyst* 51, 327 (1926); C. S. King and G. Etzel, *Ind. Eng. Chem.* 19, 1004 (1927); D. L. Drabkin and C. S. Waggoner, *J. Biol. Chem.* 89, 51 (1930); Lillian W. Conn, Arnold H. Johnson, H. A. Trebler and V. Karpenko, *Ind. Eng. Chem., Anal. Ed.* 7, 15-23 (1935).

these standards. Store the xanthate reagent in an amber glass-stoppered bottle and prepare every few days.

Copper by Urobilin

Urobilin reacts with cupric ion to give a yellow to red or purple color.[134] The sensitivity of the method in neutral or slightly alkaline solution permits determinations as low as 0.001 mg. by microtechnics. The color changes are such that the series-of-standards technic is desirable. The sensitivity of the reaction can be lowered by making the solution slightly acid with acetic acid or with mineral acid and then adding sodium acetate in slight excess. The color is not affected by aluminum, calcium, barium, ammonium, cadmium, cobalt, stannous, beryllium, lithium, magnesium, manganese, nickel, gold, strontium, bismuth, silver, arsenate, arsenite, molydate, tungstate, or vanadate ions. Ferrous ions intensify the color. Zinc causes a green fluorescence. Mercury interferes but can be corrected.

Procedure. For micromethods transfer a 1-ml. aliquot to a small tube. Take a series of standards in similar tubes, using a copper solution containing 0.005 mg. of copper per ml. for their preparation. To the standards add the same amounts of the same reagents present in the final sample.

If mercury is present render the sample and standards faintly acid with 1:10 sulfuric acid. Then add 1 drop of 1 per cent potassium iodide solution to the acid solution. A precipitate of mercuric iodide is formed. Dissolve it with 0.2 ml. of 1 per cent sodium thiosulfate solution. Add the same amounts of potassium iodide and sodium thiosulfate solutions to standards.

Carefully neutralize sample and standards to litmus with 1:10 ammonium hydroxide. Add 1 drop of 1:10 ammonium hydroxide. Add 0.4 ml. of a solution containing 5 mg. of urobilin per 100 ml. of redistilled alcohol. Dilute to 2 ml. The color develops at once.

Copper by Rubeanic Acid

Rubeanic acid, dithiooxamide, develops an olive-green color with copper in acetic acid solution, which is suitable for colorimetric estima-

[134] A. Emmerie, *Chem. Weekblad.* **27**, 552-4 (1930); Gabriel Bertrand and Louis de Saint Rat, *Compt. rend.* **203**, 140-3 (1936); Pierre Thomas, *Biochem. Z.* **293**, 396-8 (1937).

tion.[135] From ammoniacal solution copper, nickel, and cobalt are quantitatively precipitated.[136] The color precipitates unless held in colloidal dispersion hy a protective colloid such as gum arabic or gelatin. The intensity of color follows Beer's law. Bismuth does not interfere but lead, tin, antimony, and iron must he tied up in the form of complexes such as tartrates. Since the amount of iron which can he so eliminated has a limit, it is applied to ammoniacal filtrates from steel with correction for the copper retained hy the iron precipitate. The color of nickel interferes least at pH 5.2. The color of cobalt interferes at all pH levels. The reagent imparts a red color which is eliminated by use of a filter such as 650 mμ. The method is best applied photoelectrieally to aliquots containing about 0.1 mg. of copper. Thus with ores it gives results in 15 minutes accurate to 3 per cent.

Procedure. Transfer an aliquot of sample solution containing 0.05-0.4 mg. of copper to a 100-ml. volumetric flask. If mineral acid is present, approximately neutralize to methyl orange. Dilute to about 90 ml. and add 5 ml. of a huffer containing 40 ml. of glacial acetic acid and 40 grams of ammonium acetate per 100 ml. Add 1 ml. of a 0.1 per cent solution of rubeanic acid in absolute ethanol. Dilute to volume und mix. Read the transmittance within 2-3 minutes, at 650 mμ if considerable amounts of interfering ions are expected, at 400 mμ if very small amounts of interfering ions are present.

Copper by Reduced Phenolphthalein

The presence of amounts of copper as small as 0.01 ppm. can be determined with phenolphthalin, the reduced form of phenolphthalein.[137] Glycerine, cyanides, and other materials also react. The color developed is that of phenolphthalein with alkalies.

Procedure. Prepare a reagent from a solution of 2 grams of phenol-

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[135] Priyadaranjan Rây, *Z. anal. Chem.* 79, 94-101 (1926); Noel L. Allport and G. H. Skrimshire, *Quart. J. Pharm. Pharmacol.* 5, 461-72 (1932); K. Quandel, *Arch. Eisenhuttenw.* 14, 601-4 (1941); Anders Ringbom and Folke Sundman, *Finska Kemitsamfundets Medd.* 51, 42-54 (1942); Anders Ringbom, *Metall u. Erz* 40, 228-30 (1943); E. John Center and Robert M. MacIntosh, *Ind. Eng. Chem., Anal. Ed.* 17, 239-40 (1945).

[136] Priyadaranjan Rây and R. M. Rây, *Quart. J. Indian Chem. Soc.* 3, 118-26 (1926).

[137] Pierre Thomas and Georges Charpentier, *Compt. rend.* 173, 1082-5 (1921); Pierre Thomas, *Biochem. Z.* 293, 396-8 (1937).

phthalein in 100 ml. of 20 per cent potassium hydroxide solution. Add 10 grams of zinc dust, boil until completely decolorized, filter, and store in a tightly stoppered bottle. Add 4 drops of reagent and 1 drop of 4-5 volume hydrogen peroxide to 10 ml. of sample. Mix and compare with a series of standards prepared at the same time. The time for development of color may be substantial but a blank develops none in several hours.

Copper by Thiocyanate and Guaiacum

A very sensitive test for copper is by means of the blue color produced with thiocyanate in the presence of a tincture of guaiacum resin.[138] The sample will not tolerate oxidizing agents other than a small amount of hydrogen peroxide, present to insure the copper being in the form of cupric ion, and to avoid reduction by the reagent.

Procedure. Transfer a 5-ml. sample to a suitable tube and if acid, neutralize to approximately the methyl orange end point. Add 2 ml. of a 10 per cent solution of ammonium thiocyanate in methanol. Mix and add 1 ml. of 1:4 acetic acid. Add 1 ml. of a solution of 1.25 ml. of 30 per cent hydrogen peroxide per 100 ml. of methanol. Add 1 ml. of tincture of guaiac resin, mix, and dilute to 10 ml. Read the transmittance at about 570 mμ and compare with a calibration curve prepared under similar conditions.

Miscellaneous

Among the numerous colorimetric determinations carried out with 8-hydroxyquinoline is that of copper. The reagent in chloroform extracts copper, which is then determined by the yellow color.[139] Close control of pH is necessary for many metals but not for copper. Color with iron, cobalt, nickel, bismuth, and aluminum indicates the desirability of their absence. By reducing the pH below 5.5 it is applicable to copper in the presence of zinc and cadmium, having been applied at pH 4.0 in the presence of 40 times the amount of those elements. The method has been applied only spectrophotometrically but should be

[138] H. Imbert, R. Imbert and P. Pilgrain, *Bull. soc. chim.* 35, 60 (1924); R. Fleming, *Analyst* 49, 275 (1924); G. Schwarz and O. Fischer, *Milchw. Forsch.* 18, 196-204 (1936); *Proc. 11th World's Dairy Congr., Berlin* 2, 52-6 (1937); *Molkerei-Ztg.* (Hildesheim) 54, 345-6 (1940); J. Effern, *Vorratspflege u. Lebensmittelforsch.* 4, 55-8 (1941).

[139] Therald Moeller, *Ind. Eng. Chem., Anal. Ed.* 15, 346-9 (1943).

applicable with natural or artificial standards, with due allowance for the color of the reagent itself. The yellow color conforms to Beer's law up to 10 ppm.

As sample, take an aliquot containing about 1 mg. of copper. If this sample contains iron or aluminum render definitely alkaline with 1:1 ammonium hydroxide and heat nearly to boiling to coagulate. Filter and wash on the paper with hot 1:15 ammonium hydroxide until the washings show no color of copper. If the amount of precipitate is substantial, redissolve and reprecipitate. Concentrate or dilute the solution to about 25 ml. Finally cool and add 1:10 sulfuric acid until the pH is about 4.0. Extract with 5 ml. of a 0.15 per cent solution of 8-hydroxyquinoline in chloroform. Separate the extract and repeat the extraction with 3 similar solutions. Combine the 4 extracts and dilute with chloroform to 25 ml. or, usually, to 50 ml. according to the intensity of color obtained. Read the transmittance at about 410 mμ.

A reddish violet color is produced when a copper solution is treated with an oxidizing agent, silver nitrate and dimethylglyoxime, followed by aqueous pyridine.[140] Not over 0.1 mg. per 100 ml. should be present in the sample and the method will detect 0.1 ppm. Small variations in the amount of pyridine present will affect the color radically and excess causes fading. If the concentration of sulfuric acid is greater than 0.008 *N*, the coloration is faint and fugitive. Addition of more pyridine will not stabilize the color. Low sulfuric acid content gives erratic results. Buffers detract from the sensitivity of the reaction or cause too rapid fading. With less than 0.6 ppm. the color reaches a maximum in 5 minutes. Larger amounts reach their maxima and start to fade by that time.

Interference by nickel can usually be obviated by chloroform extraction.[141] Both copper and nickel compounds with dimethylglyoxime are soluble in chloroform but the nickel compound is removed by washing the chloroform extract with dilute ammonium hydroxide. This is not applicable if the nickel is in large excess over the copper.

Excess silver nitrate introduces a yellow color and promotes fading. Increase of ammonium persulfate promotes fading, probably due to acidity resulting from its decomposition. Sulfates and nitrates do not interfere. The presence of 2 mg. of sodium sulfate, magnesium sulfate, calcium sulfate, or potassium nitrate per ml. does not affect the intensity

[140] S. G. Clark and B. Jones, *Analyst* **54**, 333-4 (1929); Loren C. Hurd and John C. Chambers, *Ind. Eng. Chem., Anal. Ed.* **4**, 236-8 (1932); G. I. Alzenberg and E. M. Men'shikova, *Zavodskaya Lab.* **12**, 673-4 (1946).

[141] D. Gardner Foulke, *Monthly Rev. Am. Electroplaters' Soc.* **32**, 7-10 (1945).

or stability of the color. If the chloride content exceeds 0.005 mg. per ml., the method is unreliable. More than 0.002 mg. of iron per ml. causes serious error. Cobalt present must not exceed 0.00002 mg. per ml.

To 40 ml. of neutral sample, and an appropriate standard diluted to 40 ml., free from chlorides, add 1 ml. of 0.4 *N* sulfuric acid, 0.5 gram of ammonium persulfate, 0.5 ml. of a saturated solution of dimethylglyoxime in 95 per cent ethanol, and 0.25 ml. of 0.5 per cent solution of silver nitrate. Mix and add 1.5 ml. of a 10 per cent solution of pyridine in water. Mix well, dilute to 50 ml. and compare by balancing.

A quick comparison of 0.02-2 mg. of copper as sulfate per 100 ml. by reaction with benzidine and sodium salicylate can be made with a standard.[142] The color is stable for only 5 minutes. Alkalies and alkaline earths may be present, but silver interferes. The system does not follow Beer's law. The method has been applied by measurement of transmittance. Without a filter 0.25-5 mg. of copper are read. A green filter reduces the amount necessary to 0.01 mg. per 100 ml. By use of a red filter 1.0-50 mg. per 100 ml. are read.

If heavy metals are present, render distinctly alkaline with 1:1 ammonium hydroxide, boil for 5 minutes until only a faint odor of ammonia is present, and filter. Convert the filtrate to the sulfate by evaporation until sulfur trioxide fumes nearly cease. Dilute the sample and an appropriate standard to about 70 ml. Add 10 ml. of fresh 3 per cent sodium salicylate solution, 10 ml. of concentrated ammonium hydroxide, 2 ml. of a colorless 0.1 per cent solution of benzidine in 20 per cent acetic acid, and 2 ml. of 0.75 per cent potassium cyanide solution. Stopper the tubes to prevent loss of ammonia and mix well. Compare the color at once by balancing, or read the transmittance. An artificial standard of acidified methyl orange can also be used but fading of the color renders it less reliable than a natural standard.

Copper in steel can be determined by benzidine and thiocyanate.[143] To 5 ml. of sample solution, alkaline with ammonium hydroxide, add 2-3 pieces of marble, 1-2 mm. in diameter, and evaporate to about 1 ml. Cool and add sequentially 10 ml. of 5 per cent starch solution, 2 ml. of 0.1 per cent solution of benzidine in water, and 10 ml. of 10 per cent ammonium thiocyanate solution. Dilute to 50 ml. and read the transmittance. The color does not change significantly in 8-10 hours.

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[142] N. A. and Iv. Tananaev, *J. Russian Phys Chem. Soc.* 60, 453 (1928); *Z. anorg. allgem. Chem.* 170, 113-27 (1928); A. B. Shakhkeldian, *Zhur. Prikladnoi Khimii* 2, 475 82 (1929); Willi Maier, *Z anal. Chem.* 116, 410 21 (1939).

[143] V. M. Kopeliovich, *Zavodskaya Lab.* 11, 475-7 (1945).

but within 1 hour. Photometric methods are suitable or the sample may be balanced against a standard prepared at the same time.

When an alcoholic solution of piperidinium piperidyldithioformate is added to a solution of a copper salt, a very stable yellowish-brown color is produced.[150] This is not altered by a slight excess of acid or alkali. Cadmium, mercury, and bismuth do not interfere. Iron must be absent. Results have been successfully read in terms of Lovibond glasses.

Transfer an aliquot of the copper solution to a 10-ml. tube. Dilute nearly to volume and add 1 ml. of a 0.1 per cent solution of piperidinium piperidyldithioformate in ethanol. Dilute to volume and compare with a standard similarly treated.

The reaction of a cupric salt with potassium iodide liberates one equivalent of iodine with the formation of cuprous iodide. Other metals showing this reaction, such as ferric ion, must be absent. The iodine is then determinable in any of the usual ways.[151] The solution should be mildly acid. The reaction will detect 0.2 ppm. of copper. Originally it was based on the time before iodine color was shown with starch.

The insoluble compound of copper with ammonium tetrathiocyanodiammono-chromate, known as Reinicke's salt, can be filtered on an inorganic filter and washed. Then, dissolved in thiourea and methylethyl ketone, it is suitable for colorimetric estimation.[152] Mercury and cadmium interfere.

A rapid method [153] for copper consists of the color reaction with urea. Allowing for the nitric acid present in the sample, make it nearly 1:1 by addition of concentrated nitric acid. The volume should be about 20 ml. Add 15 ml. of 5 per cent urea solution and 10 ml. of concentrated sulfuric acid. If lead or tin is present it will precipitate and can be centrifuged out. Read the transmittance with a red filter and compare with a calibration curve.

Bi-*o*-anisidine gives a green color with copper in aqueous acetic acid solution which is suitable for colorimetric estimation.[154] Large amounts give a blue precipitate, very small amounts turn yellow after a few minutes.

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[150] Ralph G. Harry, *Analyst* 56, 736-7 (1931).

[151] H. B. Dunnicliff and K. Ram, *Kolloid-Ztg.* 38, 168-70 (1926).

[152] C. Mahr, *Angew. Chem.* 53, 257-8 (1944).

[153] C. E. Gubbins, *Metal Ind.* (London) 68, 312 (1946).

[154] E. Loriente and J. Casas, *Nature* 159, 470 (1947).

A novel technic for estimation of copper by the visual photometer is as follows.[155] React 1.5 per cent ferric chloride solution with 2.5 per cent sodium thiosulfate solution until a maximum color is attained. Add this to the sample solution and read the color at intervals at 463 mμ until it has been reduced 50 per cent. Read the time required in terms of copper from a standard curve.

[155] Hidehiro Goto and Emiko Sudo, *J. Chem. Soc. Japan* **64**, 509-14 (1943).

CHAPTER 6

CADMIUM

CADMIUM is becoming more and more industrially significant. It replaces tin in some solders, bearing metals, and alloys. It is applied as plating. This wider distribution, coupled with its volatility when heated, increases the possibility of its presence in foods and biological samples. Aside from that it is widely distributed in minerals in small amounts.

As would be expected from the similarity of properties, cadmium is determined by some of the same reactions as those for copper. The principal methods in order of relative importance are as dithizonate, by di-β-naphthylcarbazone, and as sulfide.

SAMPLES

Zinc.[1] Dissolve a 25-gram sample in 1:5 nitric acid and neutralize to litmus with 1:1 ammonium hydroxide. Add a 1 per cent aqueous solution of sodium sulfide with vigorous stirring until a substantial amount of discolored precipitate is obtained. This should contain all of the cadmium and only part of the zinc. Filter and confirm that cadmium precipitation is complete by adding a few more ml. of sodium sulfide solution to the filtrate. The precipitate should be white and the filtrate from the first sulfide precipitation may then be discarded. Otherwise repeat filtration and precipitation until no more cadmium is recovered.

Dissolve the precipitate of zinc and cadmium sulfides in the minimum effective volume of 1:5 nitric acid and evaporate to a small volume to drive off all the hydrogen sulfide and most of the nitric acid. Transfer the solution to a 100-ml. flask, dilute to volume, and take a 5-ml. or 10-ml. aliquot. Methods of removal of silver, mercury, copper, etc., are given later in this chapter.

Minerals.[2] These samples as described are for the dithizone method. Weigh 0.5 gram of pulverized sample into a platinum dish. Add 5 ml.

[1] Hellmut Fischer and Grete Leopoldi, *Mikrochim. Acta* 1, 30-7 (1937).
[2] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 11, 364-5 (1939).

of water, 1 ml. of 70 per cent perchloric acid, and 5 ml. of 48 per cent hydrofluoric acid. Evaporate to dryness and let cool somewhat. Add 0.5 ml. of 70 per cent perchloric acid and 5 ml. of water. Again evaporate to dryness to expel all the excess of perchloric acid. Add 1 ml. of concentrated hydrochloric acid and 5 ml. of water to the residue and heat nearly to boiling. Maintain at that temperature until solubles have been extracted.

Dissolve 10 grams of sodium citrate in water, add a few drops of concentrated ammonium hydroxide, and dilute to 100 ml. Shake with 5-ml. volumes of a 0.02 per cent solution of dithizone in carbon tetrachloride or chloroform until no further pink color is extracted. This removes any trace of cadmium present as an impurity.

Add 10 ml. of the prepared sodium citrate solution and 0.1 gram of hydroxylamine hydrochloride. Add concentrated ammonium hydroxide drop by drop until neutral to litmus and 2 drops in excess. If the solution is appreciably turbid at this point, filter through a small paper and wash with water. This is a solution for determining of cadmium.

Ash the small paper at a low temperature. Grind the residue with 0.15-0.20 gram of sodium carbonate in an agate mortar, transfer to a platinum crucible, and fuse. Let cool and disintegrate the melt with 5 ml. of water. Filter insoluble matter, if present, and wash with 5 ml. of water in small portions. Wash the residue from the paper with 5 ml. of 1:4 hydrochloric acid, heat to dissolve so far as possible, and filter. The combined extracts of this small sample should not exceed 20 ml. Add 3 ml. of the prepared sodium citrate solution and a small crystal of hydroxylamine hydrochloride. Add concentrated ammonium hydroxide until the solution is alkaline to litmus, and 2 drops in excess. Either combine with the previous solution or determine cadmium separately in this solution.

Solutions Containing Lead.[3] Adjust the acidity and electrolyze the solution in the same way as for electrolytic deposition of copper and lead from steel (page 81). The lead is on the anode as lead dioxide and the cadmium on the cathode. Dissolve the deposit from the cathode with 1:4 nitric acid, the amount depending on the size of the deposit. Evaporate the solution to dryness but do not decompose the salt. Take up the residue in water and dilute to a convenient volume according to the size of the cadmium deposit dissolved.

[3] H. Lecoq, *Bull. soc. roy. sci. Liege* **11**, 614-20 (1942).

Solutions Containing Zinc.[4] Remove the metals precipitated from acid solution by hydrogen sulfide. If considerable lead is present remove the bulk of it as lead sulfate. Small amounts of iron, manganese, and chromium do not interfere.

Dilute the solution to about 200 ml. Add methyl orange indicator and neutralize with 50 per cent sodium hydroxide solution. Add 1 ml. of concentrated sulfuric acid and mix. Add 0.3-1.0 gram of granular aluminum, but not less than 5 times the amount of cadmium expected. Boil for 5 minutes, filter on a wad of clean cotton in a Pyrex funnel, and wash 5 times with cold water. Transfer the cotton and precipitate to the original beaker and add 100 ml. of water. Add 0.1 gram of aluminum to the filtrate and boil for 5 minutes. Filter this on a rapid paper, wash 5 times, and add to the first precipitate. Discard the twice-treated filtrate.

Break up the filter paper with a glass rod and add 5 ml. of 50 per cent sodium hydroxide solution. When reaction ceases, filter on a strong paper, wash most of the alkali out of the spongy metal, and return the paper and contents to the original beaker. Add to the moistened cadmium sponge and filter papers, 5 ml. of concentrated sulfuric acid, 50 ml. of saturated bromine water, and a couple of drops of bromine. Heat gently until the reaction is complete and bromine has been volatilized. Dilute to about 250 ml. and pass in hydrogen sulfide for about 30 minutes. Filter the cadmium sulfide and paper residues and wash twice with saturated hydrogen sulfide solution.

Dissolve the cadmium sulfide from the filter with the minimum feasible amount of hot 1:1 nitric acid. Wash the paper well and discard. Add 5 ml. of concentrated sulfuric acid to the filtrate and evaporate to fumes. If this shows black deposits, cool, add a few drops of concentrated nitric acid, and heat again. Finally, when the residue is colorless on heating to fumes, cool and dilute to about 50 ml. Add a drop of phenolphthalein indicator and neutralize with 50 per cent sodium hydroxide solution. Add 25 per cent potassium cyanide solution dropwise until the precipitate of cadmium hydroxide is dissolved, but avoid excess. Electrolyze as usual (page 82) to separate the cadmium on the cathode. Complete as for solutions containing lead starting at "Dissolve the deposit from the cathode. . . ."

Solutions Containing Copper, Silver, and Mercury. If necessary to remove copper, add a saturated aqueous solution of sodium carbonate

[4] F. E. Townsend and George N. Cade, Jr., *Ind. Eng. Chem., Anal. Ed.* 12, 163-4 (1940).

to the acid solution of sample until turbidity appears. Add a few drops of 1 per cent sulfurous acid to clear the turbidity. Heat the solution nearly to boiling and add about 1 ml. of the sulfurous acid for each 5 mg. of copper suspected. A moderate excess will do no harm. Heat to boiling for 1 minute and cool to room temperature. The bulk of the copper is reduced to the cuprous form. Add 5 per cent sodium acetate solution until the solution turns Congo red paper from blue to red.

If copper is absent the preceding paragraph may be by-passed. If it has been followed or if silver or mercury may be present, proceed as follows. Add sufficient 25 per cent ammonium thiocyanate solution to precipitate and redissolve the first precipitate which may be any or all of the cuprous, silver, and mercury thiocyanates. Add 5 per cent sodium acetate solution until Congo red paper will just turn from blue to red. Extract with three 5-ml. portions of a solution containing 50 ml. of pyridine per liter of chloroform. Wash the combined extracts with 1 ml. of the 25 per cent ammonium thiocyanate solution diluted with 10 ml. of water and discard the washings.

Evaporate the chloroform extract to dryness on a water bath, add 3 ml. of concentrated nitric acid, and again evaporate to dryness. Add 3 ml. of 1:200 sulfuric acid, warm to dissolve, and transfer to a separatory funnel with a few ml. of water. The bulk of the silver, mercury, and copper will have been left behind but a trace may still be retained. Extract with two 2-ml. portions of a solution of 50 mg. per liter of dithizone in carbon tetrachloride to remove such traces and discard these extracts.

The extracted sample may still contain zinc, lead, bismuth, tin, etc., in addition to cadmium. Dilute the solution to 10 ml. and add 5 ml. of 20 per cent sodium potassium tartrate solution. Add 2.5 ml. of a 25 per cent sodium hydroxide solution, checked as showing no more than a trace of yellow color with dithizone, and shake. Discard the dithizone extract and dilute the cadmium solution to a known volume for the use of aliquots.

Organic.[5] A suitable sample is 50-100 ml. of urine, 5-20 grams of blood, tissue, dried feces, etc. Transfer the sample to a Kjeldahl flask and add 20 ml. of concentrated sulfuric acid, 20 ml. of concentrated nitric acid, and several pieces of silicon carbide. Heat to gentle boiling to decompose organic matter. If a small amount of persistent char is obtained, add a few ml. of 70 per cent perchloric acid, dropwise.

[5] Donald M. Hubbard, *ibid.* 13, 915-18 (1941); J. Cholak, Donald M. Hubbard and R. E. Burkey, *ibid.* 15, 754-9 (1943).

Addition of concentrated nitric acid may also be needed if the solution is near to sulfur trioxide fumes. Repeat as necessary and finally evaporate to sulfur trioxide fumes. Let cool, dilute with water, and cool. Rinse the solution of ashed sample into a 125-ml. separatory funnel. Add 15 ml. of ammonium citrate solution, prepared to contain 400 grams per liter and adjusted with concentrated ammonium hydroxide until just alkaline to phenol red. Dilute the sample solution to 50 ml., add 2 drops of phenol red indicator solution, and concentrated ammonium hydroxide until a pink color indicative of pH 8.3 is obtained. This method was designed for determination by di-β-naphthylcarbazone.

In dry ashing of organic samples at 500° those containing over 0.01 mg. of cadmium or which deflagrate give low results. Therefore wet ashing is preferred.

Plant Tissue. A solution containing zinc, cadmium, and lead is isolated by dithizone extraction under lead (page 31). Use an aliquot with the dithizone method under conditions where zinc and lead dithizonates are not extracted.

Removal of Iron as Fluoride. This has been described for separation from copper (page 107). Modify for separation from cadmium to use approximately 1.5 ml. of concentrated hydrochloric acid per 100 ml. of sample and add a 20 per cent solution of potassium fluoride instead of solid sodium fluoride.

Standard

Standard. Dissolve 0.1 gram of pure cadmium by warming with 1:100 hydrochloric acid and dilute to 100 ml. This contains 1 mg. per ml. Alternatively dissolve 0.1631 gram of anhydrous cadmium chloride in water. Prepare successive dilutions of 10 ml. to 100 ml. with the same acid to give 0.1 mg. per ml. and 0.01 mg. per ml. The final dilution should be used within a few days.

Cadmium by Dithizone[6]

The extraction of cadmium as the red dithizonate occurs only at high pH levels, starting at pH 10 but being readily completed at pH 12.[7] It follows that cadmium dithizonate is easily decomposed, as by 0.01 N

[6] For a more detailed discussion of this reagent and precautions necessary in its use refer to page 3.

[7] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66-72 (1939).

hydrochloric acid, which hardly affects the dithizonates of copper, mercury, silver, and several other metals. In strongly alkaline solutions, as typified by 4 per cent sodium hydroxide solution, cadmium can be extracted with a solution of dithizone; but lead, zinc, and bismuth are not extracted. The presence of large amounts of zinc leads to low results in such extraction of cadmium. Copper, silver, gold, mercury, palladium, nickel, and cobalt are extracted with cadmium under these conditions. Cobalt and nickel remain behind if stannous chloride is present. Cyanide is useless for promotion of separations because it forms an unreactive complex with the cadmium. If cobalt and nickel are extracted as dithizonates, that of cobalt is not decomposed by 0.01 *N* acid but nickel dithizonate is partially decomposed.

Variability of tint renders the cadmium dithizonate difficult to read. So it is desirable to wash out excess dithizone and dithizonates of foreign metals, and then extract the cadmium, leaving the equivalent as dithizone in chloroform to read. Cadmium dithizonate decomposes fairly rapidly in carbon tetrachloride.

The extraction of cadmium with dithizone has been applied to determination of such minute traces as 0.00001 mg.[8] The final solvent may be carbon tetrachloride or, in the absence of zinc, chloroform. In some cases technics of fractional precipitation of cadmium are used, for example, hydrogen sulfide precipitation[9] at pH 3 in the presence of 2 per cent sodium citrate, with copper to serve as a collector. Cadmium pyridine thiocyanate can be extracted with chloroform to separate from silver, mercury, and cuprous ions.

Procedure. Transfer a sample containing 0.003-0.05 mg. of cadmium to a suitable separatory funnel with a few ml. of water. If a series of standards is to be used transfer them to similar funnels. Dilute such standards to the same volume as the sample with the same aqueous solvent that is present in the sample. A suitable series of standards ranges upward from 0.0005 mg. at 0.0005 mg. intervals.

Add 5 ml. of a 0.02 per cent solution of dithizone in carbon tetrachloride and shake for 1 minute. Let the carbon tetrachloride settle and draw off into another separatory funnel. Unless this solution is greenish, continue with further 5-ml. portions until the extract does show a greenish color after shaking for 1 minute. If the sample solution con-

[8] Hellmut Fischer, *Angew. Chem.* 50, 919-32 (1937); Hellmut Fischer and Grete Leopoldi, *Mikrochem. Acta* 1, 30-7 (1937); E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 11, 364-5 (1939); Franklin W. Church, *J. Ind. Hyg. Toxicol.* 29, 34-40 (1947).

[9] Lawrence T. Fairhall and Leon Prodan, *J. Am. Chem. Soc.* 53, 1321-3 (1931).

tains much zinc the solution may continue to show red on successive extractions. If an extract shaken with an equal volume of 2 per cent sodium hydroxide solution is decolorized there is no cadmium in it, extraction is complete, and that extract may be discarded. If zinc is present in the extract, shake the combined extract with half the volume of 2 per cent sodium hydroxide solution and discard the wash solution. Repeat this operation. In the absence of zinc omit this step.

Shake the combined dithizone extracts with 5 ml. of water. Discard the water layer and shake the extract with 5 ml. of 1:1000 hydrochloric acid which has previously been extracted with 0.01 per cent dithizone solution in carbon tetrachloride and washed with carbon tetrachloride. Separate the organic solvent solution and extract with another 5 ml. portion of acid. Combine the acid extracts and discard the carbon tetrachloride. Shake the extract with 1 ml. of carbon tetrachloride to separate any small colored droplets remaining in the solution and withdraw this. The loss of a drop or two of aqueous phase is permissible.

Compare the free dithizone equivalent to the cadmium promptly, either with standards or by transmittance using a filter centering around 620 mμ. If only a small amount of cadmium is present, wash the carbon tetrachloride extract with water and read at 520 mμ. In either case the colors fade and the hue changes on standing.

Alternatively, neutralize the sample and dilute or concentrate to about 5 ml. Add 5 ml. of 20 per cent sodium potassium tartrate solution and mix. Then add 10 ml. of 10 per cent sodium hydroxide solution. Extract with a solution of 40 mg. per liter of dithizone in carbon tetrachloride, in successive increments until no red color is removed. Refer to the previous procedure, the paragraph starting "If the sample solution contains much zinc . . ." Finally extract the aqueous solution with 1 ml. of carbon tetrachloride and add it to the extracts. Repeat if any red color is shown, then discard the aqueous solution. Wash the combined extracts twice with 2 per cent sodium hydroxide solution and complete as for the previous method starting at "Shake the combined dithizone extracts with 5 ml. of water."

Cadmium by Di-β-Naphthylcarbazone[10]

This is a modification of the dithizone method. In general dithizone

[10] The same precautions as to reagents and apparatus are necessary as for dithizone. Where lead is the usual contaminant in reagents for lead, zinc is the common contaminant to be removed from reagents for cadmium. For details refer to page 3.

could be substituted for di-β-naphthylcarbazone,[11] except for purification of ammonium hydroxide, but the carbazone is preferable for the initial extraction and final development of color with cadmium. Thus in contrast to dithizone, the reagent and its metal complexes are insoluble in aqueous alkali and fewer extractions are required. This would be disadvantageous when cadmium is separated from lead and zinc by extraction from strongly alkaline solution, since all metals forming complexes with di-β-naphthylcarbazone would be extracted. For that step dithizone is used. The carbazone in the final step eliminates the necessity for strict pH control.

Separation from nickel is effective by the dithizone technic specified. Excess reagent and a mixed-color photometric method are necessary to stabilize the final color.[12] Sensitivity to 0.0001 mg. up to 0.005 mg., and 0.001 mg. at 0.005-0.050 mg. is attained. Only the transmittance method has been applied.

Procedure. Transfer the sample or an aliquot to a separatory funnel. Add a 5-ml. portion of a solution containing 0.2 gram of di-β-naphthylthiocarbazone per liter of chloroform. Shake well, let separate, and draw off the chloroform layer into a separatory funnel. Repeat with successive 5-ml. portions until the color of the extract is unchanged, combining the extracts. This removes cadmium, zinc, lead, and some other metals. Discard the aqueous layer. Shake the combined chloroform extracts with 50 ml. of water and draw off the extract into a separatory funnel. Shake the wash water with 5 ml. of chloroform and add this to the chloroform layers. Discard the wash water.

Shake the chloroform layer with 50 ml. of 0.2 N hydrochloric acid, draw off the chloroform layer, and discard. Wash the aqueous acid layer with 5 ml. of chloroform to remove entrained reagent and discard the wash chloroform. The aqueous layer contains at least cadmium, lead, and zinc if present. Add 5 ml. of 25 per cent sodium tartrate solution and 20 ml. of a solution containing 25 grams of sodium hydroxide per 100 ml., and adjust the volume to 100 ml. with distilled water.

Prepare a solution containing 0.1 gram of dithizone per liter in chloroform and another of one-tenth that concentration. Put 5 ml. of the more concentrated solution in a separatory funnel. Extract the alkaline solution of cadmium with 5 ml. of the more dilute dithizone solution. Add this extract to the separatory funnel containing the more

[11] Donald M. Hubbard and S. W. Scott, *J. Am. Chem. Soc.* 65, 2390-3 (1943); Jacob Cholak and Donald M. Hubbard, *Ind. Eng. Chem., Anal. Ed.* 16, 333-6 (1944).
[12] Cf. E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 11, 364-5 (1939).

concentrated reagent and repeat the extraction with successive portions of the dilute reagent solution until the extract is colorless. Wash the combined chloroform extracts with 50 ml. of distilled water and discard the washings.

Shake the chloroform solution of cadmium with 50 ml. of 0.2 *N* zinc-free hydrochloric acid. This extracts the cadmium into the aqueous phase, free from other metals, and the chloroform layer may be discarded. Add 50 ml. of ammonium hydroxide. Add 5 ml. of chloroform containing 20 mg. of di-β-naphthylthiocarbazone per liter and shake for 1 minute. Let stand until separation is complete and dilute the chloroform layer with 20 ml. of pure chloroform. Read the transmittance of this solution with a filter having its maximum transmission at about 540 mμ.

Alternatively, if the cadmium content is too high for this treatment, make the final extraction with 5 ml. of chloroform containing 200 mg. of di-β-naphthylthiocarbazone per liter, dilute with 95 ml. of chloroform, and read.

Cadmium as the Sulfide

Estimation of cadmium as the sulfide is somewhat more difficult than that of most of the sulfides because of the rather narrow pH range in which it can be completely precipitated.[13] Differences of less than 0.1 mg. per 50 ml. are indistinguishable in ordinary light but in the mercury arc can be read as low as 0.01 mg. per 50 ml. At 0.4-1.0 mg. of cadmium per 100 grams of organic sample the average error is 5 per cent. The necessity of removing iron and lead completely may require a long and complicated preparation of the sample. The presence of substantial quantities of zinc, nickel, cobalt, and copper is permissible.

The initial color develops and reaches a constant value in about 15 minutes, and remains constant for about 24 hours. It complies with Beers law. Considerable variation in the amounts of ammonium hydroxide, ammonium salts, and excess hydrogen sulfide does not affect the result. The excess potassium cyanide must be between 200 mg. and 600 mg. per 50 ml. The same color intensity develops at 15-70°. Measurement of transmittance at 30-80 per cent absorption is accurate to about 0.6 per cent.

[13] Lawrence T. Fairhall and Leon Prodan, *J. Am. Chem. Soc.* **53**, 1321-3 (1931); George Hessel, *Biochem. Z.* **177**, 146-55 (1936); Robert Juza and Robert Langheim, *Z. anal. Chem.* **110**, 262-70 (1937); *Angew. Chem.* **50**, 255-60 (1937); V. P. Maevskaya and N. P. Komar, *Zavodskaya Lab.* **7**, 36-41 (1938).

Procedure. *Zinc, Copper, Nickel, and Cobalt Absent.* Add to the neutral or faintly ammoniacal solution of sample containing 0.2-0.5 mg. of cadmium about 1 ml. of 1:25 ammonium hydroxide and 4 ml. of a 10 per cent potassium cyanide solution. If ammonium salts are not already present in the solution from the preparation of the sample, add 1 ml. of 10 per cent ammonium sulfate solution. Finally add 1 ml. of a 1 per cent solution of gelatin and dilute to about 30 ml. If natural standards are to be used, prepare them at the same time in the same way, matching the salt content of the sample. Put 5 ml. portions of a saturated aqueous solution of hydrogen sulfide into 50-ml. tubes. To each add the 30 ml. of sample or standard, and dilute to 50 ml. If the transmittance is to be determined, use a blue filter centered at 430 mμ and a solution of sample and reagents without sulfide as comparison solution.

Zinc, Copper, Nickel, or Cobalt Present. Select the aliquot of sample and adjust to substantial neutrality. If zinc is present up to 100 times the amount of cadmium, titrate the sample with 10 per cent potassium cyanide solution until the zinc precipitate is dissolved and add 4 ml. in excess.

Apply the same technic with not more than 10 mg. of copper in the sample; more than that amount will cause turbidity. Because of the blue color introduced by copper, transmittance must be used for measurement in that case.

When nickel is present, titrate with the 10 per cent potassium cyanide solution. The solution first becomes blue, then slightly yellow. More cyanide will make it more strongly yellow. Titrate to the minimum color and add 4 ml. of 10 per cent potassium cyanide solution in excess. At a maximum, 100 times as much nickel as cadmium may be present. Cobalt acts like nickel but gives a more intense color so that not more than 10 mg. is permissible in the sample.

In case any or all of these metals are present complete the determination by the previous procedure starting "If ammonium salts are not already present . . ." In all these cases, for absorption use as blank the same solution, similarly titrated but without addition of sulfide.

Miscellaneous

Cadmium forms an insoluble compound with ammonium tetrathiocyano-diammono-chromate, Reinicke's salt This, when filtered on an inorganic filter and washed, is soluble in thiourea and methylethyl ketone.

The solution is suitable for colorimetric estimation.[14] Mercury and copper interfere.

Cadmium at 10 ppm. in nearly neutral solution gives a deep violet-blue color with a saturated alcoholic solution of diphenyl semicarbazide.[15] By care in adjustment of pH the reaction will detect 1 ppm. and may be suitable for colorimetric estimation.

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[14] C. Mahr, *Angew. Chem.* **53**, 257-8 (1944).

[15] C. F. Miller, *Chemist-Analyst* **25**, 10-11 (1936).

CHAPTER 7

BISMUTH

Bismuth is not only widely distributed in minerals, but is usually present in copper and lead alloys. It is used medicinally and is therefore often found in biological samples. Its poisonous nature makes it important in small amounts. In general, the amounts of other heavy metals in biological samples will not interfere and therefore isolation of the bismuth from them can be by-passed.

The classical method for its determination as the iodide is still important. A yellow color developed with thiourea is also significant, and the familiar dithizone reaction is widely applied. Other methods are of lesser significance.

Samples

Aluminum Alloys.[1] Dissolve a 2-gram sample in 50 ml. of 1:1 hydrochloric acid without heating. When reaction has ceased, add 50 ml. of water and filter. Wash the residue with hot water. Discard these washings but save the filtrate. Dissolve the residue from the paper with 40 ml. of hot 1:1 nitric acid, using the original beaker as receiver. Wash the paper well with hot water. Add 1 ml. of 1 per cent ferric nitrate solution, then 1:1 ammonium hydroxide until turbidity just appears in the solution. If the end point is overrun, clarify with 1:1 nitric acid and again approach carefully. Add 1 ml. of 1:1 hydrochloric acid and dilute to 250-300 ml. with hot water. Place on a steam bath for 45 minutes to coagulate bismuth oxychloride.

Add 40 ml. of concentrated nitric acid and a few beads to the original hydrochloric acid solution of the sample and evaporate to about 5 ml. Filter the oxychloride precipitates and discard the filtrates. Dissolve bismuth oxychloride from the paper with 40 ml. of hot 1:1 nitric acid, dilute to a known volume, and use an aliquot for determination of bismuth by the potassium iodide method.

The entire sample may also be present in the sample solution prepared.[2] React a 0.8-gram sample with 10 ml. of 40 per cent sodium

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[1] George Norwitz, Samuel Greenberg, and Freda Bachtiger, *Anal. Chem.* 19, 173-5 (1947).

[2] J. H. Bartram and P. J. C. Kent, *Light Metals* 9, 229-31 (1946).

nel and dilute to 200 ml., using the dilution water for the transfer and washing.

Extract the solution with successive 10-ml. portions of 0.1 per cent dithizone solution in chloroform until the extract is a clear green. This should be true of the third extract. Wash the aqueous layer with 5 ml. of chloroform and separate. Discard the aqueous layer. Wash the combined chloroform extracts with 50 ml. of water and discard these washings. Evaporate the chloroform extract nearly to dryness, add 1 ml. of concentrated sulfuric acid, and heat to strong sulfur trioxide fumes. Let cool slightly and add 2-3 ml. of 30 per cent hydrogen peroxide. Heat until this is driven off, the solution is colorless, and strong sulfur trioxide fumes are again evolved. Let cool, take up with about 15 ml. of water, and chill. Filter if lead sulfate precipitates. Dilute to a known volume and use an aliquot as sample. This technic effectively prevents interference by copper, silver, nickel, phosphorus, and arsenic.

Direct determination of bismuth in a 1-gram sample is obtained[7] by dissolving in mixed hydrochloric and nitric acids, neutralizing with 1:1 ammonium hydroxide, making definitely acid with 1:1 sulfuric acid, and adding 6 grams of potassium iodide and 5 grams of sodium hypophosphite for each gram of copper. On standing for 10 minutes, cuprous iodide is precipitated and the iodine bleached.

Traces of Bismuth. In the determination of traces of bismuth a special procedure is required.[8] Dissolve a 200-gram sample of metal in the minimum volume of 1:1 nitric acid, add a small crystal of ferric sulfate, and make ammoniacal with 1:1 ammonium hydroxide. Add small amounts of ammonium carbonate and sodium phosphate and boil. Allow the precipitate to settle and filter. Discard the filtrate and dissolve the precipitate in 1:10 sulfuric acid. Pass in a stream of hydrogen sulfide to precipitate antimony, arsenic, and bismuth as sulfides. Filter and discard the filtrate. Extract the mixed sulfides with yellow ammonium sulfide solution and again filter. Dissolve the remaining precipitate in 1:10 sulfuric acid, make alkaline with ammonium hydroxide, and add 10 ml. of 5 per cent potassium cyanide solution. Reprecipitate the bismuth with hydrogen sulfide. The copper remains in solution as the cyanide complex. Filter, dissolve the bismuth sulfide in 10 ml. of 1:1 nitric acid, and take to fumes with 6 ml. of concentrated sulfuric acid in order to remove lead as lead sulfate. Dilute with 25 ml. of water, chill, and filter. Use the filtrate or an aliquot as sample.

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[7] H. R. Fitter, *Analyst* 63, 107-9 (1938).

[8] C. O. Jones and E. C. Frost, *Ind. Eng. Chem.* 18, 597 (1926).

Alternatively,[9] dissolve a 5-gram sample in 60 ml. of 1:1 nitric acid and boil until oxides of nitrogen are evaporated. Dilute to about 200 ml., cool, and add 2 ml. of 10 per cent aluminum nitrate solution. Add concentrated ammonium hydroxide in small amounts until the copper is present as the ammonia complex. This will usually require about 28 ml. The aluminum hydroxide precipitate contains all the bismuth. Filter and wash the precipitate with cold 1:25 ammonium hydroxide. Discard the filtrate.

If the precipitate is blue-green due to sorption of copper, dissolve in 4.5 ml. of hot 1:2 sulfuric acid. Cool and add ammonium hydroxide as before. Filter and wash. Finally dissolve the precipitate in 4.5 ml. of hot 1:2 sulfuric acid and use all or an aliquot as sample for determination by the thiocyanate method.

Copper Solutions.[10] Neutralize the solution with 1:1 ammonium hydroxide. Heat to 80° and add more 1:1 ammonium hydroxide until a slight permanent turbidity of basic copper salt appears. Add 1 ml. of a 5 per cent solution of manganese sulfate. Mix and heat to boiling. Add 2 ml. of a 3 per cent potassium permanganote solution to the boiling liquid, drop by drop, stirring during the addition. Filter the precipitate of manganese dioxide, which will have sorbed the bismuth. Add another portion of manganese sulfate and repeat the addition of 1 ml. of 3 per cent potassium permanganate solution.

Dissolve the filtered precipitates in 10 ml. of 1:10 sulfuric acid by addition of hydrogen peroxide. Heat to boiling to decompose excess hydrogen peroxide and use all or an aliquot as a sample. This method of separation has been used to isolate less than 1 ppm. of bismuth in copper.

Lead.[11] For 0.005-0.01 per cent of bismuth use 2 grams of sawings or foil. For 0.01-0.30 per cent of bismuth, reduce to a 1-gram sample. Add 25 ml. of 1:9 nitric acid and heat until dissolved. Boil off the brown fumes and let cool. Dilute to a known volume and take an aliquot for the thiourea method. The presence of the lead makes this solution unsuitable for the iodide method.

For determination as the iodide add 10-12 ml. of concentrated sul-

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[9] A. I. Kokorin and I. G. Dermanova, *Zavodskaya Lab.* 12, 59-63 (1946).

[10] Kaoto Kameyama and Shoji Mokishima, *J. Soc. Chem. Ind. Japan* 36, Suppl. Binding 364-5 (1933); Bartholow Park, *Ind. Eng. Chem., Anal. Ed.* 6, 189-90 (1934).

[11] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 344-6. American Society for Testing Materials, Philadelphia, Pa.

soluble in ether. Evaporate the solution to dryness and extract the residue with 5 ml. of ether. Decant through a filter and repeat the extraction twice more. Evaporate the ether and destroy any organic matter by heating with a minimal volume of concentrated sulfuric acid to which a drop or two of 70 per cent perchloric acid is added. Take up in water as the sample.

Urine, Serum, or Plasma.[17] Transfer a sample to a Kjeldahl flask and for each 100 ml. add 10 ml. of concentrated nitric acid and 1.2 ml. of concentrated sulfuric acid. Evaporate to fumes of sulfur trioxide. Prepare a mixture of 1 part of concentrated nitric acid with 2 parts of 70 per cent perchloric acid. With the sample solution fuming add this dropwise until all color is destroyed. Add 1 ml. of 30 per cent hydrogen peroxide dropwise to the hot mixture to destroy the nitric acid, more if there is any question of this being sufficient. Boil vigorously to destroy the excess of nitric acid. Let the flask cool, add 10 ml. of water, and again evaporate to fumes of sulfur trioxide. Add 10 ml. of water and repeat the evaporation. Take this up after cooling, transfer to a volumetric flask corresponding to the bismuth content, dilute to volume, and use an aliquot as sample. If the entire sample is extracted in a 50-ml. separatory funnel the pH level will be approximately correct for determination as the iodide.

Alternatively,[18] to 10 ml. of sample in a centrifuge tube add, dropwise, 2.5 ml. of a 1 per cent solution of tricalcium phosphate in concentrated hydrochloric acid. Shake well and add 1:3 ammonium hydroxide until a distinctly basic reaction is obtained, usually about 6 ml. The precipitated calcium phosphate serves as collector for the bismuth. Centrifuge and decant the upper layer to waste. Disperse the precipitate in 5-6 ml. of water and again centrifuge, and decant. Repeat once more. Dissolve the precipitate in the centrifuge tube in 0.2 ml. of concentrated hydrochloric acid and use as sample, adding more hydrochloric acid to the sample and standard in the procedure, if necessary, to keep the phosphate in solution.

Another method of preparation[19] is to boil a 50-ml. sample with 1 ml. of concentrated ammonium hydroxide and 1 ml. of 30 per cent hydrogen peroxide. The precipitation of metals serves as a collector

[17] Reavis C. Sproull and Alexander O. Gettler, *Ind. Eng. Chem., Anal. Ed.* 13, 462-5 (1941); N. J. Giacomino, *ibid.* 7, 456-8 (1945).

[18] O. N. Zepalova, *Lab. Prakt.* (U.S.S.R.) 1939, No. 6, 24-5.

[19] F. Martillotti, *Pediatria* 44, 341-6 (1936).

for the bismuth. Filter and wash the precipitate on the filter. Discard the filtrate and dissolve the precipitate in 1 ml. of warm hydrochloric acid containing 1 part of concentrated acid to 4 parts of 30 per cent hydrogen peroxide. Use this as sample solution.

Urine samples may also be prepared [20] by boiling a 10-ml. sample with 0.4 gram of potassium permanganate and 2 ml. of concentrated sulfuric acid. When reaction is complete add 0.4 gram of oxalic acid to dissolve the manganese dioxide and cool when decolorized.

Tissue.[21] Cover a sample, such as 25 grams, with twice the volume of concentrated nitric acid. Heat gently to a clear yellow to brown solution. Let cool and if, as is usual, a layer of fat separate, filter through glass wool into a Kjeldahl flask. Rinse the previous container with concentrated nitric acid and use this to wash the fat and glass wool. Add 7.5 ml. of concentrated sulfuric acid to the flask containing the filtrate and washings and complete as for urine, serum, or plasma (page 158), starting at "Evaporate to fumes of sulfur trioxide."

Tissue and Organs without Ashing.[22] Reduce the 30 grams of sample, such as organs, to small pieces. Dilute with water if necessary. Add 5 ml. of concentrated hydrochloric acid and 5 ml. of 1 per cent cupric chloride solution Bring to a boil. Immerse a sheet of clean copper foil having an area of not less than 4 sq. cm. for every mg. of bismuth present. Boil for 1 hour with the copper completely immersed, stirring occasionally. The bismuth is displaced and deposits on the copper.

Remove the copper foil and wash with water. Dissolve in 1:1 nitric acid, using the *minimum volume for complete solution.* Add 1:1 ammonium hydroxide until a faint turbidity is obtained. Add 12 drops of 1:1 hydrochloric acid and dilute with boiling water to double the volume. Heat for 1 hour on a boiling water bath to complete precipitation of bismuth oxychloride. Filter and wash the precipitate. Dissolve the precipitate from the paper with 5 ml. of 1:3 sulfuric acid. Dilute to a volume such that the concentration is not more than 10 mg. per 100 ml. and use an aliquot.

[20] P. J. Hanzlik, A. J. Lehman, A. P. Richardson and W. Van Winkle, Jr., *Arch. Dermatol Syphilol.* 36, 725-8 (1937).

[21] Reavis C. Sproull and Alexander O. Gettler, *Ind Eng. Chem., Anal. Ed.* 13, 462 5 (1941).

[22] A. Valyashko and P. K. Virup, *Ukrain. Khem Zhur.* 5, Sci. Pt. 275 92 (1930).

Blood.[23] By this method both bismuth and arsenic are obtained in a form suitable for use. Mix 5 ml. of blood in a Kjeldahl flask with 27 ml. of concentrated nitric acid and 3 ml. of concentrated sulfuric acid. Heat and, when oxidation is substantially complete, add 30 ml. of concentrated nitric acid to complete the digestion. When the solution is colorless, cool and add 70 ml. of water. Boil to destroy traces of nitrosyl sulfuric acid and cool.

Use this as a solution for distillation of arsenic by the general method (page 183). After distillation of the arsenic, add 20 ml. of concentrated nitric acid to the residue in the flask. Boil to the appearance of sulfur trioxide fumes to remove hydrazine sulfate. Cool and add 20 ml. of water. Again heat to sulfur trioxide fumes and cool. Dilute to a known volume and take a suitable aliquot for the determination.

Plasma.[24] Transfer a sample of 10-ml., or less, to a Kjeldahl flask and add 1.8 ml. of concentrated sulfuric acid and 5 ml. of a 3:1 mixture of 70 per cent perchloric acid and concentrated nitric acid. Digest over a low flame and add a few ml. of the oxidizing mixture from time to time to avoid carbonization. After the digestate is nearly colorless, with oxidation completed, let cool, add 30 ml. of water, and heat to sulfur trioxide fumes. Let cool and add 3 ml. of water. Cool and transfer to a 10-ml. volumetric flask. Rinse down the sides of the flask with water to make the transfer quantitative and dilute to volume. Take aliquots for determination of bismuth and antimony by the iodide method.

Foodstuffs. A method of isolation of bismuth and lead as sulfides, with details of treatment of the sulfide precipitate, has been given (page 98). Use an aliquot of the solution so prepared.

Pharmaceutical Preparations.[25] If organic matter to be destroyed is absent, treat a 1-gram sample with 2 ml. of concentrated hydrochloric acid. Dilute to 100 ml. with 1:20 hydrochloric acid and filter. Use an aliquot of the filtrate as sample.

Oil.[26] Unless halogens are present, ash and take up the ash in hot 1:10 sulfuric acid. When halogens are present neither saponification or ashing gives satisfactory recovery.

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[23] Eugene H. Maechling, *J. Lab. Clin. Med.* 18, 1058-61 (1933).
[24] Evan W. McChesney, *Ind. Eng. Chem., Anal. Ed.* 18, 146-51 (1946).
[25] Zenon M. Lugones, *Rev. centro estud. farm. bioquím.* 27, 67-73 (1937).
[26] Manoel Fonseca, Jr., *Rev. brasil. farm.* 26, 129-32 (1945).

Separation of Copper, Zinc, Bismuth, Lead, and Tin. Details of the separation are given under lead (page 33). The solution as so prepared contains the bismuth and lead, free from the other metals.

Separation of Bismuth from Lead. See page 33.

Standard

Prepare a standard solution by dissolving 0.2321 gram of bismuth nitrate, $Bi(NO_3)_3 \cdot 5H_2O$, in 50 ml. of 1:10 nitric acid and dilute to 1 liter. A similar standard may be prepared by dissolving 0.1 gram of pure bismuth or 0.115 gram of bismuth oxide, Bi_2O_3, in 5 ml. of 1:1 nitric acid and diluting to 1 liter. Sufficient acid must be added to these solutions in diluting, so that the bismuth does not precipitate as the subnitrate. They contain 0.1 mg. of bismuth per ml. As a more dilute standard, dilute 50 ml. of the stock standard to 1 liter with the addition of sufficient nitric acid to keep the solution clear. Such a diluted standard contains 0.005 mg. of bismuth per ml.

Bismuth as the Iodide

A solution of a bismuth salt, on addition of excess soluble iodide, assumes a yellow to orange color usually referred to as BiI_3, which has been determined nephelometrically but is suitable for colorimetric estimation.[27] Both acid solution and a reducing agent are essential. The reaction is applicable photoelectrically with a filter around 450 mμ.[28] The Corning 554 H. R. lantern blue filter, 6.0 mm. in thickness, is also suitable, with Corning 396, light shade Aklo as heat filter. The reaction is very sensitive and therefore precautions are necessary to

[27] F. B. Stone, *J. Soc. Chem. Ind.* 6, 416 (1887); W. Autenrieth and Armand Meyer, *Munch. med. Wochschr.* 71, 601-3 (1924); C. S. Leonard, *J. Pharmacol.* 28, 81-7 (1926); B. Glassman, *Z. physiol. Chem.* 172, 300-9 (1927); J. A. Sultzaberger, *J. Am. Pharm. Assoc.* 16, 218-21 (1927); C. S. Leonard and Alma Chaplin, *Compt.-rend. Congr. Pharm. Liege* No. 47, 197-204 (1934); L. A. Haddock, *Analyst* 59, 163-8 (1934).

[28] H. Baggesgaard Rasmussen, K. A. Jackerott and S. A. Schou, *Dansk. Tids. Farm.*, 391-403 (1927); *Biochem. Z.* 193, 53-61 (1928); J. Bodnár and Anton Karell, *Biochem. Z.* 199, 29-40 (1928); W. F. Von Oettingen, *Physiol. Rev.* 10, 221-81 (1930); A. J. Lehman, P. J. Hanzlik and A. P. Richardson, *J. Lab. Clin. Med.* 21, 95-7 (1935); Julius R. Scholtz and Albert L. Chaney, *Am. J. Syphilis, Gonorrhea, Venereal Diseases* 23, 759-70 (1939); Reavis C. Sproull and Alexander O. Gettler, *Ind. Eng. Chem., Anal Ed.* 13, 462 5 (1941); C. J. Wesley Wiegand, George H. Lann and Frank V. Kalich, *ibid.* 13, 912-15 (1941).

avoid contamination from sorbed deposits on glassware. It is desirable to rinse glassware with a 1 per cent ascorbic acid solution after use of acid potassium bichromate, thus reducing any oxidizing agent not fully rinsed away.

As much as 20 mg. of iron, 2 mg. of lead, 0.5 mg. of copper, 10 mg. of arsenic, and a trace of mercury may be present per 25 ml. of sample, but 0.5 mg. of silver interferes. Nitrates, aluminum, magnesium, and zinc do not interfere. No more than a small amount of cadmium may be present as large amounts form a competitive complex. Platinum, palladium, tin, and antimony form colored compounds with iodide ion. Oxidizing agents are removed by addition of a reducing agent. Excess silver or copper will be precipitated as the silver and cuprous iodides, and the solution can be centrifuged from them without loss of bismuth. Precipitation of lead iodide results in loss of bismuth. Excessive amounts of mineral acids must also be absent. In the presence of a small amount of iron, boiling permanently darkens the color. Large amounts of alkali or ammonium salts or chlorides bleach the color.

The presence of moderate amounts of potassium or ammonium sulfate in the sample does not interfere, but as a matter of usual precaution should be present in the standard. The color developed conforms to Beer's law up to 2 mg. per 100 ml. of solution. This color is a function of the iodide concentration, which must therefore be closely controlled but is only slightly influenced by the acidity or sulfite concentration. The reagent blank is at a minimum at 2 *N* acidity. Unless some reducing agent is present there may even be precipitation of free iodine. When sulfite is used as the reducing agent, it is necessary to check in a blank determination and, if it gives a yellow color with the reagent, a solution of sulfur dioxide in distilled water may be substituted or the alternative hypophosphorous acid used as reducing agent.

The use of sulfur dioxide alone for reduction is undesirable as the iodosulfinic acid, $I(HSO_2)$, formed gives a yellow color. By use of ascorbic acid with sulfur dioxide this is avoided.[29] Ascorbic acid alone is not a sufficiently strong reducing agent. Extraction of the color for concentration is effective with a 3:1 mixture of amyl alcohol and ethyl acetate [30] and the extract follows Beer's law. The iodine liberated by iron and other oxidizing agents may also be quantitatively titrated to a starch end point with sodium thiosulfate solution, as a substitute for reducing agents shown in the procedure.[31] Glycerol is sometimes added to the solution

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[29] N. J. Giacomino, *Ind. Eng. Chem., Anal. Ed.* **7**, 456-8 (1945).

[30] L. A. Haddock, *Analyst* **59**, 163-7 (1934).

[31] O. N. Zepalova, *Lab. Prakt.* (U.S.S.R.) **1939**, No. 6, 24-5.

before development of color to avoid hydrolysis and permit the use of a lower acidity. The amount of potassium iodide added to standard and sample may be varied over a wide range. It is preferably over 1 per cent to minimize error in addition. For development of the maximum color a minimum of 0.66 per cent of potassium iodide is necessary.[32] No less than 0.001 mg. of bismuth can be estimated and for that the procedure must be judiciously modified to a micro method. With 2.5 mg. of bismuth in a 25-ml. sample, a mean deviation of ±2 per cent is usual; at 0.02 mg. of bismuth, accuracy to ±5 per cent is found. By micromanipulation and extraction for concentration, 0 001-0.01 mg. of bismuth in 10-20 ml. of original organic sample is determined within ±6 per cent. Lovibond standards are available for the range 0.01-0.2 mg. of bismuth in a 25-ml. solution.

Antimony gives the same reaction but by reading the transmittance under two different conditions the two are separately determined.[33] In this technic, ascorbic acid serves to reduce iodine liberated and to stabilize the color.

Procedure. Transfer an aliquot of sample containing 0.005-0.050 mg. of bismuth to a 25-ml. volumetric flask and, unless the transmittance is to be read, a standard to another. Add acid and salts to the standard to correspond to those in the sample.

Add 5 ml. of 1 per cent ascorbic acid solution and mix thoroughly. Add 2.5 ml. of a fresh 3 per cent solution of potassium iodide and mix. Shake and finally add 1 ml. of a fresh sulfite reagent containing 0.75 gram of sodium sulfite and 0.6 ml. of concentrated sulfuric acid per 100 ml. The color is fully developed within 10 minutes. If any cloudiness is present, centrifuge. If the container is sealed, the color is stable for at least 2 weeks.

If desirable to concentrate, extract the color by shaking with 6 ml. of 3:1 amyl alcohol:ethyl acetate solvent. Shake vigorously for 2 minutes and filter the separated solvent before reading the color. If more than 0 01 mg. of bismuth is present in the sample it is necessary to extract more than once. The color in the solvent may be read photometrically against a solvent blank, or the sample compared with a standard. The color so extracted is stable for no longer than 30 minutes

[32] Norbet Benotti and Francis M. Thurmon, *J. Investigative Dermatol.* 4, 1-6 (1941).

[33] Evan W McChesney, *Ind. Eng. Chem., Anal. Ed.* 18, 146-9 (1946).

Alternatively,[34] transfer an aliquot of sample to a 25-ml. volumetric flask and, unless a photometric method is to be used, an equivalent standard to a similar flask. Add to the standard such acids and salts as are in the sample. Dilute each to about 12 ml. To each add 5 ml. of 5 per cent potassium iodide solution and mix. At this stage ferric iron and other oxidizing impurities will oxidize iodides to iodine. To each add 5 ml. of 10 per cent hypophosphorous acid solution and mix. This will reduce the iodine although it is inadequate to reduce all the original oxidizing impurities. Dilute to volume, mix, and read the sample or compare the two. The color will not then change for several hours.

Bismuth and Antimony. Prepare two strengths of reagents. The strong reagent contains 112 grams of potassium iodide and 20 grams of recrystallized ascorbic acid per liter. This reagent keeps about a month in a brown bottle but must be discarded if molds develop. The weak reagent contains 1.6 grams of potassium iodide and 20 grams of ascorbic acid per liter. It keeps about a week. Bismuth gives 11 per cent more color with the strong reagent than with the weak one. Calibrate the photometer in terms of bismuth with both the weak reagent and the strong reagent.

To an aliquot of sample in approximately 1:5 sulfuric acid, add an equal volume of the weak reagent. This gives directly the amount of bismuth present as the concentration is not sufficient to develop the color with antimony. As blank setting use a mixture of equal parts of reagent and 1:5 sulfuric acid. Read the transmittance at 420 mμ.

To another aliquot of the sample in approximately 1:5 sulfuric acid add an equal volume of the strong reagent. This gives both antimony and bismuth. If beyond the scale of the instrument, dilute a smaller aliquot of sample with 1:5 sulfuric acid before development of color. Also correct both readings by subtraction of the yellow color shown by reading a similar aliquot of sample diluted with an equal volume of 1:5 sulfuric acid.

Read the bismuth directly from the corrected first determination. Multiply the reading by 1·11 and subtract that from the corrected second reading. The difference is antimony, to be read from the calibration curve for bismuth with the strong reagent. At the concentration of reagent used, bismuth and antimony are exact equivalents.

[34] C. J. Wesley Wiegand, George H. Lann and Frank V. Kalich, *Ind. Eng. Chem., Anal. Ed.* 13, 912-15 (1941).

Bismuth by Thiourea

When thiourea is added to solutions containing bismuth, a yellow color is produced by the formation of complex bismuth-thiourea compounds such as $Bi(CSN_2H_4)_5(NO_3)_2OH$, $Bi(CSN_2H_4)_2Cl_3$, and $Bi(CSN_2H_4)_3Cl_3$. This qualitative reaction has been developed into a quantitative method.[35] Sufficient nitric acid must be present to prevent hydrolysis of the bismuth salt without having much effect on the color produced. The sensitivity is not high, making this method more suitable for substantial amounts of bismuth than for small amounts.

In high concentrations, silver, mercury, lead, copper, cadmium, and tin give white precipitates. In more dilute solutions they give neither color nor precipitate. Copper salts are decolorized by the reagent. Antimony gives a color similar to that from bismuth. This interference can be removed by the addition of hydrofluoric acid, which forms a complex with the antimony. Interference by ferric ion is prevented by reduction to ferrous ion. High concentrations of chromium, nickel, and cobalt give colored ions. To separate bismuth from them, precipitate with hydrogen sulfide in acid solution. Impurities usually present in pig lead will not interfere. Selenium forms a precipitate and low concentrations of tellurium give an interfering color. To obtain reproducible results both the acidity and concentration of thiourea must be controlled.[36] Photometric reading at 420 mμ or 460 mμ is suitable.

The method is most satisfactory at a concentration of 0.2-4.0 mg. of bismuth per 100 ml. The accuracy is then within ± 4 per cent. The system follows Beer's law.

Procedure. Evaporate an aliquot of the sample solution containing up to 4 mg. of bismuth until acid has been substantially all removed. As little as 0.01 mg. of bismuth can be determined in 25 ml. This necessarily means that a solution of sample in sulfuric acid is undesirable, although it can be used. If the nitric acid content is known it may be adjusted to 1:15 and avoid this evaporation. Take up the moist residue with 10 ml. of 1:15 nitric acid. If ferric iron is present, add 0.1 gram of hydrazine sulfate and bring to a boil to reduce the iron. If antimony is present add 0.5 gram of solid sodium fluoride. Cool and add 10 ml. of 5 per cent thiourea solution, with accuracy to 0.1 ml. If metals are

[35] C. Mahr, *Z. anal. Chem.* 94, 161-6 (1933); *ibid.* 97, 96-9 (1934); S. L. Tompsett, *Analyst* 63, 250-2 (1938).

[36] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 345-7. American Society for Testing Materials, Philadelphia, Pa.

present which precipitate, chill, filter on an inorganic filter, and wash the filter with 1:15 nitric acid saturated with thiourea. Dilute the filtrate to 25 ml. with 1:15 nitric acid saturated with thiourea. Compare with a standard similarly prepared, or read the transmittance around 420 mμ.

The color develops at once and is stable for at least an hour. Sulfur turbidity gradually develops, and may appear within an hour. The method has also been applied with color glasses as standards.

Bismuth by Dithizone[37]

Bright orange bismuth dithizonate is extracted over the range pH 3.0-9.0.[38] At high pH levels the compound begins to decompose. The dithizone mixed-color method is therefore applicable to bismuth[39] after separation from lead.[40] The introduction of any lead with reagents added in development of color will be reported as bismuth. Bismuth is not a normal contaminant of the reagents. Tin would interfere but is oxidized and removed. Separation from lead and thallium is provided. If the original sample contains excessive amounts of calcium, iron, and phosphate ions, add 1 mg. of copper as a collector and separate lead, copper, and bismuth as sulfides by the method described (page 33) for equivalent separation for lead determination. Interference by copper is later prevented by extraction from potassium cyanide solution at a pH of 7-8.[41] Cyanide also prevents interference by cadmium, zinc, and silver. Lead, thallium, and stannous tin interfere in cyanide solution. At pH 3 bismuth can be extracted from aqueous solution leaving almost all lead in the solution. At pH 4 lead is appreciably extracted.

Bismuth forms a complex with diethyldithiocarbamate which is extractable from faintly acid solution with ether.[42] Another method of separation is as the iodide complex by extraction with ethyl acetate and amyl alcohol. By following the complex extraction procedure described recoveries of 99 per cent can be expected.

Bismuth may be separated from a lead solution before its final estimation. Extract a nitric acid solution of the two metals, which has been adjusted to pH 2.0, with the dithizone reagent. Lead remains in the

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[37] For a more detailed discussion of this reagent and precautions necessary in its use, refer to page 3.

[38] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66-72 (1939).

[39] P. A. Clifford and H. J. Wichmann, *J. Assoc. Official Agr. Chem.* 19, 130-56 (1936).

[40] Donald M. Hubbard, *Ind. Eng. Chem., Anal. Ed.* 11, 343-5 (1939).

[41] Hellmut Fischer, *Angew. Chem.* 50, 919-32 (1937).

[42] S. L. Tompsett, *Analyst* 63, 250-2 (1938).

aqueous phase and bismuth forms the chloroform-soluble complex. When applied to samples from biological materials such as blood, feces, and mixed foods containing bismuth or large amounts of inorganic salts, salts are often entrained by the chloroform in the first extraction. These interfering salts, however, can be removed by washing the dithizone chloroform extract with water.[43]

Accuracy within 5 per cent can be expected for quantities of 0.05 mg. and the method is applicable to 0.005 mg. Only the photometric method has been used for estimation. The presence of hydroxylamine hydrochloride minimizes oxidation by ferric ion. Alternatively, the saturation of the sample solution with sulfur dioxide prevents oxidation by iron and interference by tin.[44] The parallel reaction with di-β-naphthylthiocarbazone gives a magenta color.[45]

Procedure. *Separation from Copper.* Transfer a suitable aliquot of sample solution, usually containing 0.005-0.60 mg. of bismuth, to a separatory funnel and dilute to about 50 ml. Adjust to slightly alkaline with concentrated ammonium hydroxide. Add 5 ml. of 10 per cent potassium cyanide solution. Add 3 drops of thymol blue indicator and adjust the solution to about pH 9.5 with 1:9 ammonium hydroxide. Extract with successive 5 ml. portions of a solution of 20 mg. of dithizone per liter of chloroform. This removes both lead and bismuth, but leaves copper in the aqueous phase. Each 5 ml. portion extracts about 0.025 mg. of bismuth. After an extracting solution is unchanged in color, wash the sample solution with two 2-ml. portions of chloroform, and combine all the chloroform extracts.

To insure complete recovery, especially important if more than 0.05 mg. is present, add 2 ml. of concentrated nitric acid to the aqueous phase, again adjust the pH to 9.5 with 1:9 ammonium hydroxide and extract with 10-ml. and 5-ml. portions of the dithizone reagent. Add these to the previous extracts and discard the aqueous solution. Wash the combined chloroform extracts with 25 ml. of water to remove excessive entrained cyanide. Wash this wash water with two 2-ml. portions of chloroform. Add the chloroform washings to the other chloroform phase and discard the wash water.

Extract the lead and bismuth from the chloroform layer by two 25-ml. portions of 1:99 nitric acid, or more if over 0.05 mg. of lead and bismuth

[43] Karl Bambach, *Ind. Eng. Chem., Anal. Ed.* 11, 400-3 (1939).

[44] E. F. Kluchesky, B. J. Longley and F. L. Kozelka, *J. Pharmacol.* 74, 395-400 (1942).

[45] Donald M. Hubbard, *Ind. Eng. Chem., Anal. Ed.* 12, 768-71 (1940).

are present, and discard the chloroform layer. Extract the acid solution with two 2-ml. portions of chloroform to remove the last of the dithizone.

Separation from Lead. Add 3 drops of *m*-cresol purple indicator (Vol. I, p. 183), and 1:9 ammonium hydroxide to adjust to pH 2.0. Extract the bismuth from the solution with the dithizone solution in 5-ml. portions so long as color is removed. Lead remains behind. The amount of bismuth is now approximately indicated as 0.025 mg. per portion required. Combine these chloroform extracts and extract the bismuth from them with two 25-ml. portions of 1:99 nitric acid. Remove any dissolved chloroform by evaporation, either in the air or under vacuum.

Development of Color. Prepare an ammoniacal cyanide solution containing 20 grams of potassium cyanide and 150 ml. of concentrated ammonium hydroxide per liter. If contaminated with heavy metals, extract with a chloroform solution of dithizone. Add 10 ml. of this to the 50-ml. of an aqueous acid solution of bismuth and extract with an amount of reagent according to the expected amount of bismuth.

Bismuth (mg.)	*Dithizone Concentration (mg./liter)*	*Volume (ml.)*	*Length of Cell (mm.)*
0-0.005	6	10	50
0-0.025	12	25	25
0-0.050	24	25	12

Read the transmittance of the clear chloroform layer around 505 mμ. Alternatively, compare with a series of standards prepared to cover the expected range.

Bismuth as the Sulfide

When a solution of a bismuth salt of the proper acidity is treated with hydrogen sulfide in the presence of a protective colloid the yellow color of the sulfide can be used for colorimetric estimation,[46] provided interfering ions are absent. Protective colloids used include gelatin, natural gums, and polyvinyl alcohol. If the sample solution contains iron, add a complex-forming radical. The color follows Beer's law and

[46] F. Malengreau and G. Delrue, *Arch. internat. méd. exptl.* 1, 35-46 (1924); Takmaro Yamamoto, *Bull. Inst. Phys. Chem. Research (Tokyo)* 13, 1265-6 (1934); *ibid.* 16, 1312-17 (1937).

is suitable for amounts of 0.002-0.1 mg. per ml. in the final sample. For 0.2 to 0.8 mg. in the sample used, the accuracy is within ±5 per cent.

Procedure. Measure a suitable volume of sample solution into a 100-ml. Nessler tube, and an approximate equivalent of standard into another. Add the same acids and salts to the standard as are present in the sample.

To each solution add 1 gram of sodium potassium tartrate and 10 ml. of 1 per cent gum arabic solution. Dilute each to about 75 ml. and add concentrated ammonium hydroxide drop by drop until a definite excess is present, and a drop on a spot plate is alkaline to phenolphthalein. Add 0.5 ml. of 10 per cent sodium sulfide solution and mix. Dilute to 100 ml. and compare.

Bismuth by Alkaloids and Potassium Iodide

The iodobismuthate of quinine in acetone solution has a yellow to red color which has been used for colorimetric estimation[47] with accuracy to ±3 per cent. Extracted into an organic solvent the color is red.[48] Cinchonine, and probably similar alkaloids, can replace the quinine. The usual sample is one from solution of mixed sulfides in nitric acid and in a 3-ml. sample suitable amounts of bismuth are 0.001-0.025 mg. Copper gives an interfering yellow color. Lead and thallium must be absent. Other common ions do not interfere.

Procedure. Adjust the acidity of the sample to 1:10 nitric acid and use 3 ml. Measure out an equivalent standard, add the same content of salts, and similarly adjust its acidity and volume. Prepare a reducing solution containing 20 grams of sodium formate, 0.5 gram of formic acid, 3 grams of potassium iodide, and 0.4 gram of sodium sulfite per 100 ml. Add 5 ml. of this to the 3-ml. portions of sample and standard, and mix. Add a drop of a 2 per cent solution of quinine in 1:10 sulfuric acid and mix. Shake each with 1 ml. of cyclohexanone to extract the color, making the shaking equivalent so as to get equal effects of the partition coefficient in the single extraction. Separate the hexanone layers and compare. The quinine is kept very low to avoid yellow color from formation of quinine iodide. The extracted color is stable for at least 3 days when exposed to air and light.

[47] Pierre Aubry, *J. pharm. chim.* 25, 15-18 (1922); L. Couy and G. Poirot, *J. pharm. chim.* 28, 215-23 (1923); C. E. Laporte, *J. pharm. chim.* 28, 301-5 (1923); Sidney Gaye and Julio O. Castillo, *Am. J. Clin. Path., Tech. Sect.* 5, 81-2 (1941).

[48] Arnold Okáč, *Chem. Listy* 32, 27-30 (1938).

Miscellaneous

A yellow color appears in an acid solution of bismuth on addition of potassium thiocyanate[49] which may interfere in the usual method for iron. Lead may be present but large amounts of salts interfere. Acidify the sample to about 1:2 sulfuric acid in a volume of 5-10 ml. Add 2 ml. of 50 per cent ammonium thiocyanate solution and 4 drops of 20 per cent stannous chloride solution in 1:1 sulfuric acid. Stopper and shake until the pink color due to iron disappears. Read the color at 600-650 mμ.

A reddish-orange insoluble complex is formed between bismuth and *o*-hydroxyquinoline which may be used for colorimetric estimation.[50] Precipitation is prevented by addition of acetone, or the color is extracted with acetone, amyl acetate, or cyclohexanol. Solvent extraction is not necessary if 0.25 mg. of bismuth is present. For this determination, mix equal volumes of a 4 per cent solution of potassium iodide and a 2 per cent solution of *o*-hydroxyquinoline in mixed 1:1000 nitric and 1:1000 sulfuric acid. To 5 ml. of sample solution and an equivalent standard add 0.5 ml. of the reagent, and 4 ml. of a mixture of 2 volumes of acetone and 1 volume of amyl acetate. Shake and let stand. Compare the color of the solvent layers. This bismuth complex is completely extractable with chloroform in the range pH 4.0-5.2.[51] At higher pH levels the extraction is incomplete. The color follows Beer's law in the range around 395 mμ, up to 10 mg. per liter of extract.

As a micro method 0.05-0.5 mg. of bismuth can be estimated in the presence of 80 mg. of cadmium or zinc by the reaction with Folin's reagent, phosphomolybdotungstic acid.[52] Measure suitable volumes of sample solution and standard into centrifuge tubes and add 2-3 drops of a fresh 4 per cent solution of pyrogallol. Heat to about 70° and add 1:20 ammonium hydroxide until a distinct turbidity is visible. Heat just to boiling and add 2 drops of a 0.1 per cent solution of thymol blue in 20 per cent ethanol. Add 1:20 ammonium hydroxide until an alkaline reaction is obtained with the indicator. The total volume should be about 1.5 ml. Heat for 10 minutes in a boiling water bath to precipitate bismuth tannate. Let cool, dilute to about 5 ml., and centrifuge for 10 minutes at 2000-2500 rpm. Decant and stir up the precipitates with 5

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[49] H. Heinrichs and M. Hertrich, *Glastechnische Ber.* **2**, 112-15 (1924); *Chimie & industrie* **14**, 696 (1925); J. Hubert Hamence, *Analyst* 62, 18-23 (1937); A. I. Kokorin and I. G. Dermanova, *Zavodskaya Lab.* **12**, 59-63 (1946).

[50] M. Teitelbaum, *Z. anal. Chem.* **82**, 366-74 (1930); R. Sazerac and J. Pouzergues, *Compt. rend. soc. biol.* **109**, 79-82, 370-1 (1932).

[51] Therald Moeller, *Ind. Eng. Chem., Anal. Ed.* **15**, 346-9 (1943).

[52] M. Teitelbaum, *Z. anal. Chem.* **82**, 366-74 (1930).

ml. of water. Centrifuge again and decant. If necessary, repeat the washing, using 2 ml. of water. Dissolve the precipitates in 1-ml. portions of 1:2 hydrochloric acid and dilute to about 15 ml. Add to each 1 ml. of Folin's reagent (page 623) and 6 ml. of cold saturated sodium carbonate solution. Dilute to 25 ml. and compare after 30 minutes.

In dilute solution, addition of sodium stannite to a bismuth salt gives a dispersion suitable for nephelometric estimation.[53] For development add 5 ml. of hot, filtered 0.5 per cent agar solution to 10 ml. of sample and an equivalent standard. Prepare a sodium stannite solution by adding 20 per cent sodium hydroxide solution to 20 per cent stannous chloride solution until the precipitate just redissolves. Add an equal volume of this to sample and standard and compare the resulting turbidities.

An indirect method[54] precipitates the bismuth as the phosphate and, after washing carefully, determines the phosphate by conventional methods.

A red colloidal dispersion given by bismuth with dimercaptothiodiazole is suitable for colorimetric estimation.[55] For the color development, add to 5 ml. of a slightly acid solution 5 ml. of 1:15 nitric acid, 5 ml. of 0.5 per cent gum arabic solution, then a 0.5 per cent solution of reagent until the maximum color is developed. Dilute to 25 ml. and read against standards similarly prepared. A corresponding phenyldithiobiazolone gives a yellow color,[56] suitable for estimation of 0.012 mg. of Bi per 100 ml. Colors of copper, cobalt, and nickel interfere. Another technic is turbidimetric estimation as the bromide by addition of a potassium bromate-bromide mixture.[57]

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[53] Luigi Malossi, *Rend. acad. sci. (Napoli)* **2**, 83-90 (1932).

[54] M. I. Tarasenko and V. I. Petrashen, *Izvestia Novocherkarsk. Ind. Inst.* **6**, [20], 69-74, (1940); *Khim. Referat. Zhur.* **4**, No. 3, 54 (1941).

[55] Anil Kumar Majumdar, *Science and Culture* **7**, 458-9 (1942); *J. Indian Chem. Soc.* **21**, 240 4 (1944).

[56] Anil Kumar Majumdar, *J. Indian Chem. Soc.* **21**, 347-51 (1944).

[57] Anil Kumar Majumdar, *ibid.* **21**, 157-8 (1944).

CHAPTER 8

ARSENIC

ARSENIC OCCURS naturally in many ores, and thereafter in the metals refined from them. It is also widely distributed in natural organic materials and used in some medicinals. Around 100,000 tons per year are converted to insecticidal sprays and some parts of this occur as spray residues on foods. Some types of glass contain it as one of the ingredients. All of this adds up to the necessity for determination of arsenic in a great variety of materials, often to establish conformity to legal standards.

The classical Gutzeit method, standard with tho Association of Official Agricultural Chemists, is relatively simplo to operate and gives a reasonable degree of accuracy. There are various refinements on this method. The more recently developed forms of the molybdenum blue method are more sensitive and more accurate, provided that phosphorus and silica are absent. Fortunately for avoiding interference, the isolation of arsenic is not difficult.

The methods of preparation of sample are necessarily further subdivided to this extent. The preparation of a sample in solution with the arsenic retained is the first step. This is suitable for the Gutzeit method, provided that salt concentrations are not unduly high. Often for application of other methods, arsenic is separated from it by the second step of distillation of arsenic trichloride. The volatility of trivalent arsenic halides precludes evaporation of solutions high in hydrochloric acid without loss of arsenic.

SAMPLES

Carbon Steel, Cast Iron or Ferromanganese.[1] Dissolve a 0.1-gram sample in 10 ml. of 1:1 nitric acid. Evaporate to dryness at not over 130°. Cool and take up with 5 ml. of concentrated hydrochloric acid. Use this for distillation as the chloride by the second method (page 185) starting at "Transfer to the distilling apparatus shown in Figure 11, using. . . ." When adding nitric acid to evaporate to dryness, use only 10 ml.

[1] Clement J. Rodden, *J. Research Natl. Bur. Standards* 24, 7-11 (1940).

High Chromium Steel. Dissolve a 0.1-gram sample in 5 ml. of concentrated nitric acid and 5 ml. of concentrated hydrochloric acid. Evaporate, cool, and take up with 5 ml. of concentrated hydrochloric acid. Use this for distillation as the chloride by the second method (page 185) starting at "Transfer to the distilling apparatus shown in Figure 11, using. . . ."

Ferrous Alloys.[2] Dissolve 0.1 gram in 10 ml. of 1:1 nitric acid, or, if high in chromium, in a mixture of 5 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid. After solution is complete, evaporate to dryness with the temperature held below 130°. Cool and take up with 5 ml. of concentrated hydrochloric acid. Use this for distillation as the chloride by the second method (page 185) starting at "Transfer to the distilling apparatus shown in Figure 11, using. . . ."

Brass, Bronze, and Bearing Metals. Dissolve a 0.1-gram sample in 5 ml. of concentrated nitric acid. Cool and add 8 ml. of 1:1 sulfuric acid. Evaporate to sulfur trioxide fumes and cool. Take up in 5 ml. of water and use this for distillation as the chloride by the second method (page 185), starting at "Transfer to the distilling apparatus shown in Figure 11, using. . . ."

Alternatively,[3] dissolve in a centrifuge tube. Add a 1 per cent solution of ferric sulfate dropwise, until the arsenic is completely precipitated as ferric arsenate. Centrifuge to separate the precipitate. Decant as completely as possible and wash with a few ml. of 1:1 nitric acid. Again decant and dissolve the residue in 5 ml. of 1:10 sulfuric acid.

Copper. *Electrolytic Separation.*[4] Dissolve a 10-gram sample in a mixture of 1:5 sulfuric acid and 1:5 nitric acid. Boil until oxides of nitrogen are eliminated and add 10 grams of tartaric acid dissolved in water. Dilute to about 350 ml. and electrolyze until copper is nearly absent. Remove the electrodes and wash as usual.

Add concentrated ammonium hydroxide to the solution until it is neutral to litmus and then add 3-4 ml. of 1:1 hydrochloric acid for each 100 ml. of the solution. Precipitate the sulfides by passing in hydrogen sulfide, and let settle. Filter, wash the sulfides on the paper, and discard the filtrate.

[2] *Ibid.*

[3] Yu. Yu. Lur'e. *Trans. VI Mendeleev Congr. Theoret. Applied Chem.* 1932, Pt. 2, 348-51 (1935).

[4] Bartholow Park, *Ind. Eng. Chem., Anal. Ed.* **12**, 97-8 (1940).

Transfer the filter to a beaker and heat with 5 ml. of concentrated nitric acid to dissolve the mixed sulfides. Dilute with about 40 ml. of water and filter. Concentrate the filtrate to about 5 ml. to remove much of the acid and use as sample or dilute to a known volume and take a suitable aliquot.

Lead and Lead Alloys.[5] This method separates arsenic, antimony and tin from the lead. They can then in turn be separated from each other for analysis. Weigh a suitable milled sample, such as 50 grams, into a 500-ml. conical flask and add 200 ml. of 1:3 nitric acid. Heat gently until the sample is dissolved, and add 10 ml. of 2 per cent potassium permanganate solution to oxidize the arsenic, antimony, and tin to their higher valences. Then add 20 ml. of 10 per cent manganous nitrate so that manganese dioxide may serve as a collector in precipitation, and boil gently for about 2 minutes. Filter through a rapid paper while still hot and wash the precipitate on the paper to remove lead nitrate as completely as possible. Save the filtrate and transfer the paper with the precipitate to the original flask. Add 15 ml. of concentrated sulfuric acid and 35 ml. of concentrated nitric acid, and boil gently until the paper is destroyed.

Dissolve 35 grams of ammonium sulfate in 100 ml. of water and add slowly with stirring to the hot lead nitrate filtrate. Let cool and filter on a close paper on a Büchner funnel. Refilter if the solution is not clear and wash the precipitated lead sulfate once or twice with ice water. Discard the precipitate. Add concentrated ammonium hydroxide to the filtrate until any copper present shows the clear blue color, or until alkaline to litmus if copper is absent, and 15 ml. in excess. Heat to boiling, add 10 ml. of 10 per cent ammonium persulfate solution, and boil for a minute. The metals are now present as hydroxides. Decant the solution through a rapid paper and wash the precipitate onto the paper with 3-4 portions of hot water. Some precipitate may be washed in the flask adhering to the side and need not be transferred. Discard the filtrate and add the paper to the flask in which one paper has been decomposed.

Add 35 ml. of concentrated nitric acid and boil gently to decompose the second paper. Continue to heat until all nitric acid has been volatilized and copious white fumes of sulfur trioxide are evolved. All lead sulfate should dissolve. A residue at this stage indicates incomplete washing of the precipitated hydroxides, and may be corrected by adding more sulfuric acid until solution is complete.

[5] C. L. Luke, *ibid.* 15, 626-9 (1943)

Let cool, add 0.1 gram of hydrazine sulfate and 3 grams of potassium acid sulfate, and wash down the sides of the flask with 10 ml. of water to insure all the hydrazine sulfate being in solution. Heat to copious fumes of sulfur trioxide, thus driving off the water, and continue for about a minute to be sure that sulfur dioxide has been elimiuated. Cool, add about 10 ml. of water, again cool to 25°, and use as sample for distillation as the trichloride (page 183). Start "For this transfer the sample . . .", noting that reducing agent is already present.

Alternatively,[6] dissolve a 5-gram sample by boiling with 100 ml. of 1:3 nitric acid. Cool, add 30 ml. of 1:1 sulfuric acid and evaporate to sulfur trioxide fumes. When cool, take up with 20 ml. of water. Use this for distillation as the chloride by the second method (page 185) starting at "Transfer to the distilling apparatus shown in Figure 11, usiag. . . ."

Silicate Minerals.[7] Fuse 0.5 gram of 100-mesh sample with 1 gram of sodium carbonate admixed with 2 per cent of sodium nitrate. Heat the cooled melt with water and disintegrate it. Add 2.5 ml. of 70 per cent perchloric acid and 5 ml. of 48 per cent hydrofluoric acid. Heat to a pasty condition, take up in water, and evaporate until fumes of perchloric acid cease. Take up the residue in 25 ml. of water, add 5 ml. of concentrated hydrochloric acid, and heat to about 80°. The solution should be nearly clear. Depending on the nature of the mineral it may or may not be necessary to separate the arsenic (page 183 et seq.). Results are reported as low. This is probably from loss in driving off the perchloric acid.

Talc.[8] Boil a 30-gram sample for 0.5 hour with 60 ml. of concentrated nitric acid, during which about half the volume should be driven off. Add 30 ml. of water and filter. Add 25 ml. of concentrated sulfuric acid to the filtrate and evaporate on a sand bath to sulfur trioxide fumes. Continue to heat for 10 minutes and let cool. Add 0.5 gram of hydrazine sulfate and heat to fumes for 20 minutes longer. Cool, take up in 100 ml. of water, transfer to a 200-ml. volumetric flask, dilute to volume, and use an aliquot.

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[6] Clement J. Rodden, *J. Research Natl. Bur. Standards*, 24, 7-11 (1940).

[7] E. B. Sandell, "Colorimetric Determination of Traces of Metals," pp. 145-6. Interscience Publishers, Inc., New York, N. Y. (1944).

[8] S. T. Volkov, *Mineral Syr'e* 12, No. 7-8, 48 (1937); *Khim. Referat. Zhur.* 1, No. 4-5, 172 (1938).

Pyrites and Other Sulfides. Grind the sample to pass a 100-mesh screen. Heat 100 grams of concentrated nitric acid in a 300-ml. dish on the steam bath. Weigh out a 10-gram sample. Add this in small portions to the hot acid. Stir thoroughly between additions as long as brown fumes are evolved. When half the sample has been added, increase the nitric acid by 25 ml. and continue. After the reaction is complete add 15 ml. of concentrated sulfuric acid and evaporate to sulfur trioxide fumes. Add 5 drops of 30 per cent hydrogen peroxide and stir vigorously. When the hydrogen peroxide has decomposed the sample is ready for addition of reagents for distillation of arsenic as the tribromide (page 186).

Sulfur.[9] Boil a sample of 3-5 grams with 50 grams of sodium sulfite heptahydrate and 80 ml. of water for 2-3 hours to dissolve. Add 3 more grams of sodium sulfite and then add saturated potassium permanganate solution in small amounts to the boiling solution. About 120 ml. will usually suffice. The sulfur is oxidized to sulfate and precipitation of sulfur should not occur. After 10 minutes boiling add 10 ml. of concentrated sulfuric acid and evaporate to sulfur trioxide fumes. Let cool, take up in a suitable volume of water, and use an aliquot.

Soils.[10] Transfer a 5-gram air-dried sample to a Kjeldahl flask and mix with 20 ml. of concentrated sulfuric acid. Add 5 ml. of concentrated nitric acid and 0.1 gram of potassium chlorate. Heat gently, and gradually increase to boiling. If necessary to complete the destruction of organic matter, add more concentrated nitric acid. Cool and add about 50 ml. of water. Heat to copious fumes of sulfur trioxide. Repeat this operation twice more to be sure all nitrosylsulfuric acid is decomposed. Take up in water, dilute to 100 ml., and use an aliquot for determination by the Gutzeit method.

Baking Powder.[11] Use the Gutzeit method, introducing a 5-gram sample in the generator. Add water until reaction ceases, then 15 ml. of concentrated hydrochloric acid dropwise so long as foaming occurs. Heat on a steam bath until the starch has been hydrolyzed and no

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[9] S. Yu. Fainberg and G. A. Taratorin, *Zavodskaya Lab.* 9, 1223-6 (1940).

[10] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, p. 10. Association of Official Agricultural Chemists, Washington, D. C. (1945).

[11] *Ibid.*, p. 215.

longer gives a blue with iodine. Dilute to about 30 ml. and complete as usual.

Gelatin.[12] Heat a 20-gram sample with 75 ml. of 1:3 hydrochloric acid until the gelatin is dissolved and insoluble matter flocculated. Add an excess of saturated bromine water, usually about 20 ml., then 1:1 ammonium hydroxide until neutral to litmus. Add 2 grams of disodium phosphate dodecahydrate, or of sodium ammonium phosphate tetrahydrate, and let cool. Add 30-35 ml. of magnesia mixture (page 182) and let stand for 30 minutes. This precipitates the arsenic with magnesium phosphate as collector. Filter and wash thoroughly with 1:15 ammonium hydroxide. Drain well and dissolve in 1:3 hydrochloric acid. Transfer to a 50-ml. volumetric flask and dilute to volume. The suitable aliquot for the Gutzeit method is 25 ml. In running a blank include the phosphate.

Bones. Powder the dried sample. Add an excess of 1 per cent sodium hydroxide solution and mix well. After digestion overnight, decant and repeat the extraction. Filter the first extract, then the second. Wash the residue well with water and use the combined filtrates as sample. Before use neutralize the aliquot with 1:1 hydrochloric acid.

Alternatively,[13] dissolve the sample in fuming nitric acid of d. 1.5, then dilute with an equal volume of water and extract the fat twice with ether. Evaporate the ether and separately digest the extracts (page 178). Combine these digestates with the previous one and filter the precipitated calcium sulfate. Cool and add 10 ml. of concentrated sulfuric acid. Heat to sulfur trioxide fumes. Continue as for the organic samples (page 179) starting at "Let cool and add 75 ml. of water, and . . ."

Water.[14] Make a 1-10 liter sample alkaline with sodium peroxide and evaporate to dryness. Take up this residue for distillation as trichloride (page 183) or tribromide (page 186). The American Public Health Association standard sets a maximum at 0.05 ppm. in potable water but 1-2 ppm. for several days, and 5 ppm. for a day is not dangerous.[15]

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[12] *Ibid.*, p. 387.

[13] James A. Sultzaberger, *Ind. Eng. Chem., Anal. Ed.* **15**, 408-10 (1943)

[14] W. O. Robinson, H. C. Dudley, K. T. Williams and Horace G. Byers, *ibid.* **6**, 274-6 (1934).

[15] C. C. Ruchhoft, O. R. Placek and Stuart Schott, *Public Health Reports* **58**, 1761-71 (1943).

Phosphoric Acid. Use directly as sample but allow for the acidity of the phosphoric acid and, in the Gutzheit method, omit addition of potassium iodide.

Organic Samples. A number of methods may be applied to organic samples, varying according to the nature of the sample. When wet digestion methods are used, low-temperature or oxidizing conditions must be maintained.[16] When charring of the samples takes place, arsenic may be reduced from the pentavalent to the trivalent form. The vapor pressure of arsenious oxide is substantially zero below 250°, 32 mm. at 275° and 144 mm. at 338°, the boiling point of sulfuric acid. If heated to vigorous fuming while reducing material is present, substantial loss may occur. This is avoided by keeping the temperature low until oxidation is complete. Difficultly oxidizable substances must be taken care of by dry-ashing. Pyridine derivatives, such as those from tobacco, give satisfactory results by that method. For dry ashing it is permissible to add magnesium nitrate but not cerium nitrate, as less than 20 per cent of the arsenic is recovered with the latter.[17] Wet ashing is simpler and shorter.[18]

Wet Ashing. Select a suitable weight of 5-50 grams of miscellaneous samples according to the degree of dehydration and arsenic content expected.[19] For fresh fruits carrying spray residues use 500-2000 grams and peel representative samples. Use representative samples of dried fruits, or small fruits and vegetables. Put the sample in one or more Pyrex Kjeldahl flasks and add 25 ml. of concentrated nitric acid. When the initial reaction subsides, add 20 ml. of concentrated sulfuric acid. Place such flasks over an asbestos mat with a 50-mm. opening and heat gently, withdrawing the heat if foaming becomes excessive. Thereafter in heating, rotate at intervals to avoid deposition of carbon on the area of glass receiving the direct flame. Whenever the contents of the flask become brown or darken add more concentrated nitric acid. It is essential that nitric acid be in excess at all times when organic matter and halogens are present as otherwise the arsenic may be reduced to the trivalent form and volatilized as the trihalide. Continue until the

[16] Roe E. Remington, E. Jack Coulson and Harry von Kolnitz, *Ind. Eng. Chem., Anal. Ed.* 6, 280-1 (1934).

[17] C. C. Cassil, *J. Assoc. Official Agr. Chem.* 20, 171-8 (1937); H. J. Wichmann, *ibid.* 20, 165-71 (1937).

[18] Paul Nitsche, *Pharm. Zentralhalle* 80, 701-6 (1939).

[19] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, p. 437. Association of Official Agricultural Chemists, Washington, D. C. (1945).

mixture is water-white or straw color and evolves sulfur trioxide copiously. Let cool and add 75 ml. of water and 25 ml. of saturated aqueous ammonium oxalate solution. Again heat to sulfur trioxide fumes to eliminate nitrosyl sulfuric acid. Cool, take up in water, and transfer to a volumetric flask, such as 500-ml. or 1 liter. Cool and dilute to volume. In use of an aliquot for the Gutzeit method allow for the sulfuric acid present. If interfering substances such as pyridine, excessive amounts of salts, etc., are present it may be necessary to distill the arsenic as trichloride (page 183) or tribromide (page 186) for that method.

Arsenic may also be isolated from the digestate as the sulfide, along with antimony, mercury, etc. For this, use the entire solution or an aliquot. Pass in hydrogen sulfide until precipitation is complete, cover, and set aside for an hour. Filter and wash with 1:100 sulfuric acid saturated with hydrogen sulfide.

Dissolve the arsenic and antimony from the precipitate on the filter with 10 ml. of yellow ammonium sulfide solution. Filter and discard the residual sulfides. Evaporate the filtrate to dryness on the water bath. Dissolve the residue in a few ml. of 1:1 nitric acid, evaporate to dryness, and add 3 ml. of concentrated sulfuric acid. Heat on a sand bath until sulfur trioxide fumes appear and let cool. Dissolve in water, dilute to a convenient volume, and use all or an aliquot. In use allow for the sulfuric acid present.

Where extreme precautions are necessary condense the vapors evolved during digestion, separately evaporate with nitric acid, and add to the main sample. Loss of 0.3 per cent from 4 mg. of arsenic was so demonstrated.[20] A chloroform extract containing copper and arsenic was set aside from a technic for lead (page 28).

Digestion with potassium sulfate, copper sulfate as catalyst, and concentrated sulfuric acid is suitable for foods contaminated with war gases,[21] a method analogous to the Kjeldahl method. An alternative is extraction with alkali hydroxide solution.[22]

Dry Ashing. A detailed technique for combustion with an enclosed torch and washing of the combustion gases with suitable absorbing liquids has been developed [23] for estimation of iodine (page 730). The same technic is applicable to arsenic by substitutions as indicated.

[20] Donald M. Hubbard, *Ind. Eng. Chem., Anal. Ed.* 13, 915-18 (1941).

[21] H. Amphlett Williams, *Analyst* 66, 228-33 (1941).

[22] W. J. Stainsby and A. McM. Taylor, *ibid.* 66, 233 9 (1941).

[23] Harry von Kolnitz and Roe E. Remington, *Ind. Eng. Chem., Anal. Ed.* 5, 38-9 (1933).

Sprayed Foliage.[26] Digest the sample with 1:10 nitric acid or 1:5 hydrochloric acid for 30 minutes, filter, and wash the residue. This solution will give practically the same results as by complete digestion to destroy the organic matter.

Malt.[27] For accurate results it is necessary to wet-ash, but 71-83 per cent of the arsenic is obtained by direct use of the Gutzheit method with 10-25 grams of malt.

Hops, Grain, and Sugar.[28] Place 12 grams of sample, 150 ml. of water, and 5 ml. of 20 per cent magnesium nitrate solution in a casserole. Boil gently, evaporate to dryness, and ignite carefully until free from carbon. When cool, dissolve soluble material in 20 ml. of 1:10 hydrochloric acid and 1 drop of 20 per cent stannous chloride solution. Heat on a water bath for 5 minutes, cool, dilute to 50 ml., and filter. Dilute the filtrate to a known volume and use aliquots for the determination.

Tobacco.[29] The presence of pyridine derivatives makes a special method of separation of arsenic necessary, which method is applicable to other samples containing pyridine.

Heat 200 grams of tobacco with fuming nitric acid until organic matter is completely decomposed and all chlorides expelled. Evaporate repeatedly with sulfuric acid until all nitric acid is removed. Cool, dilute with water, and when cold dilute to a standard volume. Transfer an aliquot of the diluted digested solution, representing about 0.2 mg. of arsenic trioxide, to a 400-ml. beaker and dilute to about 200 ml. Add concentrated ammonium hydroxide until distinctly alkaline. Add 20 ml. of a 1:50 dilution of 85 per cent orthophosphoric acid and mix well. Slowly add 25 ml. of a magnesia mixture containing 55 grams of hydrated magnesium chloride, 55 grams of ammonium chloride, and 88 ml. of concentrated ammonium hydroxide per liter. Stir during this addition. Add 5 ml. of concentrated ammonium hydroxide, stir and let stand for at least 15 minutes. Magnesium phosphate and arsenate are coprecipitated. Filter and wash the precipitate on the paper 4 times with 15-ml. portions of 1:9 ammonium hydroxide. Finally wash with 10 ml. of

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[26] J. M. Ginsburg, *J. Econ. Entomol.* **21**, 588-92 (1928).

[27] A. D. Comrie and T. J. Ward, J. Inst. Brewing **34**, 530-3 (1928).

[28] *Ibid.*

[29] H. Popp, *Z. angew. Chem.* **41**, 838-9 (1928); cf. C. C. Cassil, *J. Assoc. Official Agr. Chem.* **22**, 319-20 (1939).

water. Drain for 15 minutes with occasional shaking to promote complete filtration.

Dissolve the precipitate in 40 ml. of 1:4 hydrochloric acid, adding the acid in small portions. Collect the solution in a 100-ml. volumetric flask. Wash the filter with 50 ml. of water and dilute to volume. Use 5 to 20 ml. aliquots for the determination, making allowance for the acid already present.

Food Dyes.[30] This method is applicable to all certified food colors. Moisten 10 grams of dye in a Kjeldahl flask with water, then add 20 ml. of concentrated sulfuric acid and 10 ml. of concentrated nitric acid. When the initial reaction subsides, heat to drive off oxides of nitrogen and continue to add 2-5 ml. portions of concentrated nitric acid until the bulk of the organic matter is in solution. Add 1 ml. portions of 60 per cent perchloric acid, waiting until the reaction subsides each time, until 10 ml. has been so added. Then continue addition of concentrated nitric acid in small amounts. If this is not complete in 10-20 minutes, repeat the addition of perchloric acid. After the solution is colorless, boil for 10-15 minutes, cool, and transfer to a 50-ml. volumetric flask. Dilute to volume and use an aliquot.

Oils. Absorb a suitable sample in arsenic-free cotton and proceed as for combustible materials by dry ashing (page 179).

Separation of Arsenic

In general arsenic may be separated from other elements by distillation as the trichloride or tribromide, or by volatilization as arsine Precipitation methods as magnesium arsenate or as the phosphate have been given as integral parts of several methods of preparation of samples.

Distillation as the Trichloride. Select an inorganic sample, dry ash, or portion of solution low in water content prepared by digestion or other means, to contain 0.02-5 mg. of arsenic. Since this is a reduction, nitric acid must be absent but sulfuric acid does no harm.

For this transfer the sample containing a minimum of water to the apparatus as shown in Figure 10, using a small volume of concentrated

[30] "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," Sixth Edition, pp. 288-9. Association of Official Agricultural Chemists, Washington, D. C. (1945).

hydrochloric acid to facilitate the transfer. Add a few grains of silicon carbide to prevent bumping. If the sample has not already been reduced, it is necessary to add reducing agent. Usually this is 0.1-1 gram of hydrazine sulfate. That reagent must be used if the contents of the distillation flask are later to be used as a sample for determination of lead. An alternative to hydrazine is 2 grams of hydrated ferrous sulfate and 0.2 gram of potassium bromide. Cuprous chloride may also be used. The presence of bromide facilitates reduction so that addition of 0.2 gram of potassium bromide or 0.5 ml. of concentrated hydrobromic acid is desirable but not essential with hydrazine sulfate.

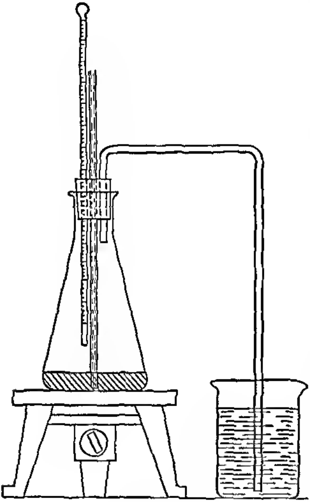

Fig. 10
Apparatus for Distillation of Arsenic as the Trichloride

Add 50 ml. of concentrated hydrochloric acid to the flask and close with a rubber stopper previously soaked in hot 1:3 hydrochloric acid. In many cases a condenser is used but it is not essential. Arsenic trichloride will not distill from a solution containing less than 16 per cent of hydrogen chloride, therefore use 300 ml. of water in a 400-ml. beaker as receiver without cooling, the gases from the side arm being led directly into this as absorber. Other designs provide one or more wash bottles, containing in some cases alkaline solutions, bromine, etc. Naturally the less the amounts of arsenic being determined the greater the precautions necessary. Distil at a moderate rate until the temperature of the solution reaches 105°, then remove from the hot plate and detach the distillation head. Germanium distills with the arsenic but does not interfere in most methods. Antimony would partially distill from this solution

above 107°. Use the distillate, suitably diluted and aliquoted, as the sample for arsenic determination. Antimony and tin remain in the distillation flask and may be isolated by application of suitable separation procedures such as distillation as antimony trichloride and as stannic chloride.[31]

Alternatively,[32] use hydrochloric acid with a minor amount of hydrobromic acid for distillation. The sample should be anything up to 25 ml. which may be low in acidity. Transfer to the distilling apparatus shown in Figure 11, using 50 ml. of concentrated hydrochloric acid for the transfer. Antimony must be substantially absent. Add 10 ml. of concentrated hydrobromic acid.

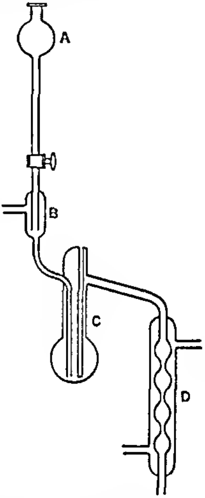

FIG. 11

All Glass Distilling Apparatus. (John A. Scherrer, *J. Research Natl. Bur. Standards* 16, 253-9 (1936), *ibid.* 21, 95-104 (1938).

In the apparatus, A is a 50-ml. bulb for making additions. The tube to B is about 25 cm. in length to overcome pressure in the apparatus when making additions, and not over 3-4 mm. in diameter to avoid drainage of the part in B. The unit at B for gauging the flow of liquid from A has a side tube for introduction of gases. C is a 200-ml. distilling flask. The neck is 2.5 cm. in diameter. Aside from the side tube leading to the bulbed condenser, D, it has an inlet tube for introducing solutions or gases near the bottom of the flask, and a sealed-in thermometer well. Both of these latter extend to within 3 mm. of the bottom of the flask. The exit tube to D is 17 cm. above the bottom.

Insert a thermometer in the well of the distilling flask and provide an ice-cooled receiver containing 40 ml. of water into which the condenser dips. Add 2 ml. of concentrated hydrobromic acid. Dissolve 1.0 gram of hydrazine sulfate in 10 ml. of concentrated hydrochloric acid. Start the passage of carbon dioxide at 2-3

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[31] W. Heuss, *Rev. tech. Sulzer*, 1945, 110-14.

[32] Clement J. Rodden, *J. Research Nat'l. Bur. Standards* 24, 7-11 (1940); Donald M. Hubbard, *Ind. Eng. Chem., Anal. Ed.* 13, 915-18 (1941). Cf. Harold J. Magnuson and Emily B. Watson, *ibid.* 16, 339-41 (1944).

bubbles per second through the apparatus and add this latter solution. Boil the solution gently until the temperature reaches 111°. This should take about 15 minutes and about 10 ml. of solution should remain in the distilling flask. Remove the receiver and distillate before turning off the heat. Add 30 ml. of concentrated nitric acid to the distillate and evaporate to dryness. Heat in an oven at 130° for an hour to remove all traces of nitric acid. Use this as sample by the alternative method for development as molybdenum blue (page 198).

Distillation as the Tribromide.[33] A broadly applicable procedure has been developed which is useful with such diverse materials as soil, digestates of organic samples, material isolated from pyrites, etc. It provides at the same time for isolation of selenium or germanium if present.

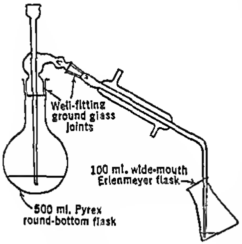

Fig. 12
Apparatus for Distillation of Arsenic

Transfer the sample, such as 50 grams of air-dried soil, to the distillation flask of the apparatus shown in Figure 12. Add 10 ml. of a solution of 10 ml. of bromine in 100 ml. of concentrated hydrobromic acid, a few ml. at a time, with shaking. This avoids loss by frothing if carbonates are present. Continue addition of this solution until it is in excess, the amount depending on the amount of organic matter in the sample. Add concentrated hydrobromic acid to bring the volume of this reagent up to 75-100 ml The greater volume is used if much carbonate is present. The hydrobromic acid must be of a grade which will be completely decolorized by sulfur dioxide.

Connect the flask with the condenser Place 2-3 ml. of saturated bromine water in the receiver with the adapter dipping below its surface. The first few ml. of distillate should also carry over a couple of ml. of bromine. If that amount does not distill over, add more to the flask. Excess bromine is harmful only in producing an excess of sulfuric acid in a later operation when destroyed by reduction with sulfur dioxide. Heat gently at first and finally with full heat until 30-50 ml.

[33] W. O. Robinson, H. C. Dudley, K. T. Williams and Horace G. Byers, *Ind. Eng. Chem., Anal. Ed.* 6, 274-6 (1934).

of distillate have collected. Add 50 ml. more of the bromine in hydrobromic acid and repeat the distillation. A third distillation may be necessary, unless experience shows that all of the arsenic is in the first distillate.

Pass sulfur dioxide into the distillate until the color of the bromine has been destroyed. Add about 0.5 gram of hydroxylamine hydrochloride. Stopper the flask loosely and heat on a steam bath for an hour. Let stand overnight at room temperature to precipitate the selenium as a red or black precipitate. Filter the selenium on an inorganic filter and wash with concentrated hydrobromic acid containing a trace of hydroxylamine hydrochloride. Reserve the residue on the filter for later estimation of selenium. Arsenic and germanium are present quantitatively in the filtrate from the selenium Add 10 ml of concentrated nitric acid and evaporate to about 25 ml. Cool, add 5 ml of concentrated sulfuric acid and heat to sulfur trioxide fumes. When cool dilute with water and use as sample for arsenic and germanium.

Volatilization as Arsine. Criticisms of the Gutzeit method usually relate to nonuniformity of the stains. It volatilizes all of the arsenic in the absence of lead and mercury, with possible interference by antimony under limited conditions.[34] Therefore volatilization as arsine is applied as a method of isolation, with subsequent absorption in sodium hypobromite solution. Large amounts of heavy metals must not be present but moderate amounts of iron are tolerated. One technic for isolation is to generate the arsine and decompose in a hot quartz tube, subsequently dissolving the arsenic in concentrated nitric acid.[35] Another technic is absorption in mercuric chloride solution and subsequent oxidation with bromine.[36] The mercuric chloride content of sample and standard must be uniform. The arsine is also absorbed in silver sulfate solution [37] and in acid mercuric chloride containing excess potassium permanganate.[38] Acid potassium permanganate, ceric sulfate, or potassium bromate alone are not satisfactory for absorption.

Transfer the digested sample to the bottle of a Gutzeit apparatus as usual in that method The absorption apparatus is shown in Fig.

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[34] Morris B. Jacobs and Jack Nagler, *ibid* 14, 442-4 (1942).

[35] *Herman J. Morris and Herbert O. Calvery, ibid.* 9, 447-8 (1937); *J. Wyllie, Can. Pub. Health J* 32, 81 2 (1941).

[36] Paul Riou and Jean P Paré, *Lab. l'école hautes études commerciales* (Montreal), Contrib. No. 17, 5 15 (1942); *Ann. l'Acfas* 8, 77-8 (1942).

[37] Gert Taubmann, *Arch. exptl. Path. Pharmakol.* 176, 751-6 (1934).

[38] E. B Sandell, *Ind. Eng. Chem., Anal. Ed.* 14, 82-3 (1942).

ure 13. To the glass heads in the absorber add 3 ml. of sodium hypobromite prepared by mixing 1 volume of 2 per cent sodium hydroxide solution with 3 volumes of half-saturated bromine water. Proceed as usual until the arsine has been completely evolved and absorbed. Transfer the contents of the absorber, wash with six 2-ml. portions of water, and dilute to volume for the use of aliquots. Antimony, bismuth, selenium and phosphates do not interfere with this separation when introduced into the generator. Use the method of development as molybdenum blue.

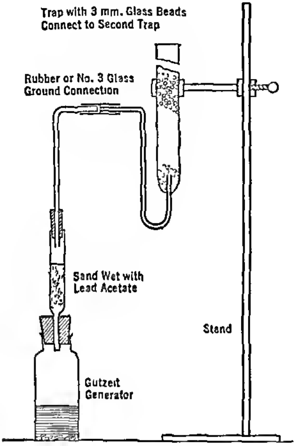

Fig. 13
Apparatus for Isolation of Arsenic From Arsine

Extraction as the Xanthate.[39] Arsenious xanthate is extractable from aqueous acid solution by carbon tetrachloride. Antimony and many other metallic xanthates are concurrently extracted. The others are washed out by concentrated hydrochloric acid without affecting trivalent arsenic. Thus lead, mercury, cadmium, aluminum, manganese, and zinc do not interfere.

Evaporate the carbon tetrachloride solution to dryness and dissolve the residue in bromine water. Boil off the excess bromine and dilute to a known volume for use of the molybdenum blue method.

Separation of Arsenate, Phosphate, and Silicate as Molybdate Complexes.[40] The molybdate complexes can be successively extracted in amounts of under 10 mg. total. Adjust the pH of the sample to ap-

[39] A. K. Klein and F. A. Vorhes, Jr., *J. Assoc. Official Agr. Chem.* **22**, 121-30 (1939).

[40] R. I. Alekseev, *Zavodskaya Lab.* **11**, 123-34 (1945).

proximate neutrality, transfer to a separatory funnel and dilute to about 100 ml. Add 1 ml. of 1:2 nitric acid and mix. Add 6 ml. of 10 per cent ammonium molybdate solution and mix. After 5 minutes add 10 ml. of 1:2 nitric acid and 5 ml. of butanol. Shake until the butanol dissolves, nearly saturating the solution. To separate phosphate add 15 ml. of a 1:3 mixture of butanol and chloroform, and invert the funnel 15-20 times. Separate the solvent and repeat the extractions until only a colorless extract is obtained. Combine the extracts, dilute to a known volume, and use an aliquot of the extract for determination of phosphate.

Add to the aqueous solution 15 ml. of 1:1 butanol-ethyl acetate mixture and shake vigorously. Add 15 ml. of chloroform and extract by inverting as before. Repeat the extractions with successive portions until the extract is colorless. Dilute these extracts to a known volume with butanol and use an aliquot of the extract for reading molybdenum blue as a measure of arsenic as arsenate. Compare the reading with a calibration curve developed under similar conditions.

To extract the remaining color, which will be due to silicate, add 10 ml. of 1:2 nitric acid and 15 ml. of butanol. Invert 15-20 times and remove the butanol layer. Repeat until the butanol extracts are colorless. Dilute the combined extracts to a known volume and read colorimetrically.

Separation of Selenium, Tellurium and Arsenic.—Remove gold, platinum, and palladium from neutral or slightly acid solution by mercurous chloride, and filter. Acidify the filtrate to make the concentration 1:4 with respect to hydrochloric acid. Add about 5 per cent of sodium bisulfite and let stand for 15 minutes. Boil gently for a few minutes to precipitate selenium Filter, dissolve in 1:10 hydrochloric acid containing free chlorine, and heat to drive off free chlorine. Use this as sample for estimation of selenium

Remove the tellurium from the filtrate by addition of mercurous chloride, warming if necessary to hasten reaction, and use the filtrate for estimation of arsenic by raising the hydrochloric acid concentration to 30 per cent.

Standard

For a standard in terms of arsenious oxide use 1 gram, in terms of arsenic use 1.32 grams. Dissolve in 25 ml. of 20 per cent sodium hydroxide solution to convert to sodium arsenite. Saturate the solution with carbon dioxide to convert the remaining sodium hydroxide to sodium

bicarbonate and dilute with recently boiled water to 1 liter. Each ml. contains 1 mg. of standard.

For a standard containing 0.1 mg. per ml. dilute 50 ml. to 500 ml. For 0.01 mg. per ml. dilute 50 ml. of this to 500 ml., and for 0.001 mg. per ml. dilute 50 ml. of this to 500 ml. Prepare these diluted standards every few days.

Arsenic by the Gutzeit Method

In determination by the Gutzeit method the arsenic is reduced to arsenious acid and then, by treatment in a hydrogen generator, to arsine. The arsine is led over paper treated with mercuric chloride or better yet with mercuric bromide and the stain produced compared with stains produced by standard amounts of arsenic.[41] Wet-ashing of plant materials is unsatisfactory unless followed by distillation of the arsenic as the chloride. Reduced sensitiveness in the presence of mercury, bismuth, copper, iron, and selenium is also avoided by distillation of the arsenic as the chloride.

The presence of pyridine derivatives in the sample causes results to be 5 to 50 per cent low due to failure of tin to adhere to the zinc used for evolution, resulting in slow evolution of hydrogen, and incomplete conversion of the arsenic to arsine. A method of separation of the arsenic to avoid such interference is given as applied to tobacco (page 182).

There are numerous modifications, most of which are inapplicable by the average analyst. These include holding the strip in a standardized position or modification to a disc and electrolytic generation of hydrogen.[42] Another variant is to pass the arsine through a filter paper wet with gold chloride.[43]

The paper strip has been modified to use a No. 8 knitting cotton with sensitivity to 0.00001 mg. and determination of 0.0001 mg.[44] Further

[41] Charles R. Sanger and Otis F. Black, *J. Soc. Chem. Ind.* 26, 1115-23 (1907); "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," Sixth Edition, pp. 436-9. Association of Official Agricultural Chemists, Washington, D. C. (1945).

[42] For earlier references see Vol. I, 2nd Edition; Henri Griffon and Jacques Thuret, *Bull. soc. chim.* (5) 5, 1129-42 (1938); A. V. Nikolaev, *Voprosy Pitaniya* 8, No. 4, 68-72 (1939); A. I. Shtenberg, *Voprosy Pitaniya* 9, No. 3, 64-73 (1940); Arnold E. Osterberg and Walter S. Green, *J. Biol. Chem.* 155, 513-18 (1944).

[43] F. Martin and J. Pien, *Bull. soc. chim.* 47, 646-54 (1930); Karl Hinsberg and Manfred Kiese, *Biochem. Z.* 290, 39-43 (1937).

[44] Alfred E. How, *Ind. Eng. Chem., Anal. Ed.* 10, 226-32 (1938).

refinements include operation under vacuum, photographic recording of results, and photoelectric comparison of the densities of prints. This requires a blank of less than 0.00004 mg. and has a limit of sensitivity of 0.00001 mg.[45] A variant is use of silver citrate photographic paper, protected from light, as the test paper.[46] The use of a small amount of stannous chloride in the generator serves to sensitize the zinc and to absorb any iodine liberated. Copper is an alternative catalyst.[47] The arsenic may be prereduced by heating in dilute acid solution with 0.05 gram of sodium bisulfite.[48] More than a trace of fluoride must be eliminated from the sample.

While granular zinc is usually supplied, the same accuracy is obtainable with stick and various size granules if all conditions are properly standardized. It is important to standardize both size and shape of the zinc used.[49] Iron is sometimes used instead of zinc in the generator to produce the desired arsine but not the undesirable stibine or phosphine. Granular aluminum has also been used. With hydrochloric acid alone its rate of evolution of hydrogen is uneven, but stannous chloride renders it uniform. The amount of stannous chloride required is greater than when zinc is used. An alternative is to etch the aluminum with 3 per cent mercuric chloride for a couple of minutes.[50]

The lead acetate conventionally used on cotton or glass wool absorbs any hydrogen sulfide evolved. It is essential that the temperature of the evolution and absorption apparatus be the same in preparation of sample and standards to maintain the moisture content of the absorbents uniform. A lower temperature in the generator will produce shorter and more intense colors. Lower temperatures in the absorption apparatus produce faint, long stains which are less definite.

Standards keep for six months if the bromide is used but will fade after a week or ten days if the chloride is used They can be satisfactorily reproduced as colored figures.[51] For very small amounts of arsenic there are variations between the standards obtained for different sets of apparatus.[52] If the stain produced has been contaminated with antimony it will be longer and lighter in color. This is confirmed by

[45] Henry S. Satterlee and Gertrude Blodgett, *ibid.* **16**, 400-7 (1944).
[46] L. Truffert, *Ann. fals.* **31**, 73-85 (1938).
[47] George Taylor and J. Hubert Hamence, *Analyst* **67**, 12-13 (1942).
[48] W. A. Davis and J. G. Maltbie, *ibid.* **61**, 96-100 (1936).
[49] N. I. Goldstone, *Ind. Eng. Chem., Anal. Ed.* **17**, 797-9 (1946).
[50] G. B. Zil'berman and K. N. Polikarpova, *Zavodskaya Lab.* **4**, 760-2 (1935).
[51] L. Longfield Smith, *Fla. Quart. Bull.* **42**, No 2, 27-33 (1933).
[52] Paul Riou and Jean P. Paré, *Ann. l'Acfas* **7**, 76 7 (1941).

exposing the stain to the fumes of hydrochloric acid, when a stain from antimony will fade but an arsenic stain will be intensified. The inherent error in application to quantities of 0.01-0.03 mg. of arsenic is 5-10 per cent.[53] The standard procedure detects 0.00001 mg. of arsenic in the sample. One serious problem is to obtain uniform sensitization of the paper.

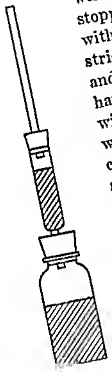

Fig. 14
Generator to be used with Gutzeit Method for Determination of Arsenic

Apparatus.[54] This is shown in Figure 14. Use 50-ml. wide-mouthed bottles as generators. Fit each by a rubber stopper to a glass tube 1 cm. in diameter and 6 cm. long with a constricted end to facilitate assembly. In the constricted end of this tube place a small wad of glass wool and add about 4 grams of 30-mesh sand retained on 40-mesh, having the same amount in each tube. Moisten the sand with 10 per cent lead acetate solution and remove the excess with a light suction. This should be uniformly moist in all cases to minimize channeling. It causes formation of shorter, deeper-colored, sharper stains. When necessary, clean the sand without removing from the tube by washing with concentrated nitric acid followed by a water wash, retreatment with 10 per cent lead acetate solution, and suction.

Connect this tube by a rubber stopper with a narrow glass tube about 2.6 mm. in diameter and 12 cm. long to contain the strip of test paper. A tube as large as 3 mm. will permit the paper to curl and give uneven stains. The tube must be dry before insertion of the mercuric bromide paper.

Test Paper. To prepare this, dry a heavy close-textured, unsized paper 1 hour at 105° and keep in a desiccator until needed. Irregular texture will result in inaccurate results. Just before use, cut into strips exactly 2.5 mm. wide and over 12 cm. long. Uniformity of width is essential to accurate results. Groups of strips may be left attached at one end. Saturate with a 5 per cent solution of mercuric bromide in 95 per cent ethanol. Attenuated stains can be modified by increase in concentration of mercuric bromide, too short stains by use of a weaker solution. After sensitizing,

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53 O. C. Cassil, *J. Assoc. Official Agr. Chem.* 21, 199-200 (1938).

54 "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," Sixth Edition, pp. 436-9. Association of Official Agricultural Chemists, Washington, D. C. (1945).

remove and dry individual strips on glass rods, or groups by waving in the air. When nearly dry press between clean sheets of paper to flatten. Store in a dark, dry place and use within 2 days. For use trim the ends to 12 cm. length before inserting in the tubes.

Procedure. *With Standard Test Paper.* Place the prepared strips of test paper in the narrow tubes, well centered. Run a blank on the reagents, which should not exceed 0.001 mg. of arsenious oxide. Titrate an aliquot of the sample so that the amount of mineral acid is known. Place an aliquot of the sample containing 0.01-0.03 mg. of arsenious oxide, and preferably 0.02-0.025 mg., in the generator. At the same time transfer standards containing 0.010, 0.020, and 0.030 mg. of arsenious oxide to similar generators. If the acid present is sulfuric, add sufficient 25 per cent sodium hydroxide solution to neutralize it and an equivalent amount of sodium sulfate to the standards. Dilute to 30 ml. and add 5 ml. of concentrated hydrochloric acid. If the sample already contains hydrochloric acid add sufficient concentrated acid to make the total volume present to 5 ml. and dilute to 35 ml. with water. Cool, if necessary, and add 5 ml. of 15 per cent potassium iodide solution and 4 drops of a 40 per cent solution of stannous chloride in concentrated hydrochloric acid. Let stand for 30 minutes at 25° or heat to 90° for 5 minutes. Cool and dilute to 40 ml.

Add to each standard or sample, at 25° or below, about 15 grams of stick zinc or 5 grams of granulated zinc. The stick zinc may have been activated by immersion in 1:3 hydrochloric acid to which a small amount of stannous chloride has been added. The purpose is to obtain uniform rates of evolution in standards and sample. Connect the apparatus as described so that the gas evolved will be filtered through glass wool and sand wet with lead acetate solution before passing over the sensitized mercuric bromide paper. Immerse the generators to within 25 mm. of the top of the narrow tube in a water bath at 20-25° and let stand for 1.5 hours.

Remove the strips and average the length on both sides in mm. Compare those from the samples with those of standards. Plotting length of stain against amount of standards is permissible and more accurate. To report in grains per pound multiply ppm. by 0.007.

Microdetermination with Thread.[55] For this method to obtain lengths of stain of the order of 6 mm. from 0.001 mg. of arsenic the AOAC method is modified as follows.

[55] Sister Emily Cahill and Sister Louisella Walters, *Ind. Eng. Chem., Anal. Ed.* 14, 901 (1942).

1. Instead of an impregnated sand scrubber, soak glass beads in saturated lead acetate overnight, pour on filter paper, and half-fill the scrubber tube. After each run wash the beads and tube first with water, 4 times with concentrated hydrochloric acid, and 4 times with water. Repeat the overnight soaking in saturated lead acetate solution, pour on filter paper, and half-fill the scrubber tube.

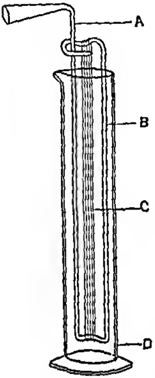

FIG. 15
Device for Impregnating Threads

A. Handle
B. Device for impregnating
C. Thread
D. Glass cylinder containing mercuric bromide.

2. Replace the paper strips with No. 24 cotton thread impregnated with the device shown in Figure 15. The cylinder is of 50-ml. capacity, the thread drawn loosely, yet sufficient to separate the strands. Fill the cylinder to within 1 cm. of the top with 4 per cent mercuric bromide solution, and impregnate for 15 minutes. Remove the frame and dry horizontally on a glass support without contact with the thread. When dry cut the impregnated lengths with scissors, handle *only* at the upper end with metal forceps, and place in the capillary at once.

3. Replace the detector tube with a 1 mm. capillary. Attach the lower end to a vacuum line to draw in the impregnated thread, which will not have been touched other than at the upper end since impregnation.

Results at 18-20° for 1.5 hours show a variation of stain for 0.001 mg. of arsenic from 5.0-7.5 mm. A No. 8 thread similarly shows a range of 4.0-7.0 mm.

ARSENIC BY THE ELECTROLYTIC GUTZEIT METHOD

Amounts of arsenic of the order of 0.001-0.1 mg. *in digested biological samples* are determinable by an electrolytic modification.[56] Even down to 0.0001 mg., estimation within 10 per cent accuracy is possible.

The apparatus is shown in full in Figure 16. Joints are lubricated with petrolatum. Both cathode and anode are bent of platinum sheet as shown. The glass wool is impregnated with lead acetate by soaking

[56] A. E. Osterberg, *J. Biol. Chem.* 76, 19-22 (1928); Arnold E. Osterberg and Walter S. Green, *ibid.* 155, 513-18 (1944).

in a 10 per cent solution for several hours, pressing out the excess, and air drying. The water-vapor chamber contains 2 ml. of water to insure saturation on contact with the prepared string.

As indicator wind 20-50 feet of No. 8 knitting cotton in closely spaced spirals on a glass cylinder. Immerse in a solution of mercuric chloride of the desired concentration. The concentration of mercuric chloride used is varied according to the sample, for up to 0.002 mg., 0.25 per cent; up to 0.01 mg., 1 per cent, above that 5 per cent. The electrolytic apparatus is believed simpler to operate when large numbers of samples are to be run. After 1 hour or longer, remove, draw off the string, and rotate in a horizontal position for 5 minutes to dry. Cut into 12 cm. pieces with a minimum amount of handling and store in a covered dark container. Each lot should be separately standardized.

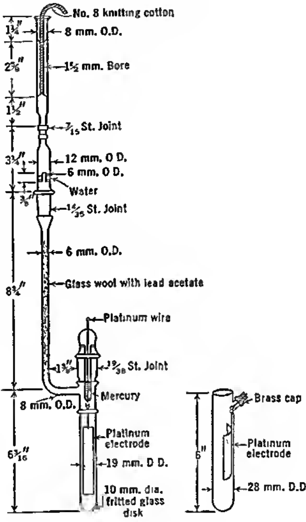

FIG. 16
Diagram of Electrolytic Gutzeit Apparatus

Procedure. Transfer an aliquot of the sample to a flask and add 2 Pyrex beads and 3 drops of 60 per cent stannous chloride solution containing 60 ml of concentrated hydrochloric acid per 100 ml. Boil for 3 minutes and cool. Fill the anode chamber with 1:7 sulfuric acid until half full and insert the cathode chamber (left) in the anode chamber (right). After 1-2 ml. of the acid has diffused through the porous disc, transfer the treated sample to the cathode chamber. Use 1-2 ml. of water to complete the transfer. The fluid level in the anode chamber should now be about 1 ml. above that in the cathode chamber. Insert the cathode with its stopper,

The reaction gives a greenish-blue at 0.0015 mg. and is considered sensitive to that limit. Lower concentrations can be detected but not accurately estimated. Over 0.050 mg. of arsenic gives too deep a blue for accurate estimation. The preferred method of determination is by reading the transmittance.

Alternative.[66] Transfer a suitable aliquot to a conical flask. If nitric acid is not already present, strongly acidify with it. Evaporate almost to dryness on a hot plate adjusted to about 120-125°. Above 140° loss of arsenic becomes appreciable. Complete the evaporation in an oven at 120° for 1 hour.

Prepare a reagent by dissolving 1 gram of ammonium molybdate in about 45 ml. of distilled water. Add 50 ml. of sulfuric acid containing 36 ml. of concentrated acid per 100 ml., and dilute to 100 ml. This solution usually gives no blank for 2 weeks. As a fresh reagent to be prepared daily dilute 20 ml. of the concentrated reagent to about 90 ml., add 2 ml. of 0.15 per cent hydrazine sulfate solution, dilute to 100 ml., and mix well.

Add 10 ml. of the fresh dilute reagent to the evaporated sample, cover, and heat in a water bath at 80-90° for 10 minutes. Cool under the tap and read the color in a 1-cm. cell with a photoelectric colorimeter with a 625 $m\mu$ filter, or dilute and compare with a standard simultaneously developed. Correct a reading of transmittance for a blank determination and in preparation of a standard be sure to use the same amounts of reagents. It is practicable to obtain a zero blank. By operating photoelectrically at 840 $m\mu$ the sensitivity is doubled.

Arsenic as the Sulfide

Provided arsenic has been separated from other metals giving insoluble sulfides under the test conditions, it can be estimated as the colloidal sulfide.[67] The arsenic present should be in the range of 0.1-1.0 mg. A protective colloid such as gelatin or gum arabic is necessary. A variation, when sufficient arsenic is present, is to filter the arsenious sulfide precipitated in acid solution, wash well, redissolve in ammonium hydrox-

[66] James A. Sultzaberger, *Ind. Eng. Chem., Anal. Ed.* 15, 408-10 (1943).

[67] Fernando V. M. Gandy and Mario P. Antola, *Anales. asoc. quim. argentina* 24, 164-5 (1936); *ibid.* 25, 76-80 (1937); Fernando Gandy, *ibid* 26, 13-20 (1928); *Bull. soc. chim. biol.* 20, 1102-7 (1938); Victor Mertens, *J. pharm. Belg.* 23, 497-502, 529-32 (1941).

ide, and add silver nitrate solution. The resulting colloidal silver sulfide is thus measured as an indirect method.[68]

Procedure. Use a sample from which other sulfides precipitating in acid solution are absent. If necessary this may have been by distillation as the trichloride, and subsequent oxidation. Usually it is a digestate.

Remove excess nitric acid from a suitable aliquot of sample by adding 30 per cent hydrogen peroxide dropwise and boil off the excess. Evaporate to dryness and take up in 5 ml. of water. Add 0.1-0.15 mg. of hydrazine sulfate and heat to boiling to eliminate sulfur dioxide. Dilute to about 12 ml. and add 3 ml. of concentrated hydrochloric acid and 4 ml. of 5 per cent gum arabic solution. Similarly treat comparable standards. Mix well, add 3 ml. of saturated aqueous hydrogen sulfide solution to each, dilute to volume, and mix. Let stand for 15 minutes and compare.

Arsenic by Sodium Hypophosphite

This nephelometric method depends on the reduction of arsenic compounds with sodium hypophosphite in strongly acid solution, Bougault's reagent, to give a dispersion of elemental arsenic.[69] This reagent also reduces organic substances such as sugars, gums, starch, and dextrins to glucose under favorable conditions, at the same time forming a brown coloring which is probably humic acid. The method is therefore applicable only in the absence of organic matter. The minimum concentration of arsenic is 0 0005 mg. per ml The arsenic dispersion can be stabilized as a solid hydrosol with silicic acid and thus kept for several months.[70]

Reagent. To prepare Bougault's reagent dissolve 20 grams of sodium hypophosphite in 20 ml. of distilled water, add 200 ml of concentrated hydrochloric acid, and filter through cotton to separate sodium chloride crystals. Leave in a cool place until a second deposition of sodium chloride occurs and filter to give a clear solution.

Procedure. To a 5 ml. sample and to 5 ml of a suitable standard solution, add 5 ml. of the reagent. Put the two tubes into a boiling water bath for 30 minutes, cool, and compare in a nephelometer.

[68] D. B. Yokhel'son, *Ukrain. Khem Zhur.* **9**, 344-7 (1934).

[69] M. Delaville and J. Belin, *Bull. soc. chim. biol* **9**, 91 (1927); V. P. Shredov, *Lab. Prakt.* (U.S S R.) **14**, No. 11, 28 30 (1939); B. S Tsyvina and B. M. Dobkina, *Zavodskaya Lab.* **7**, 1116-20 (1938).

[70] J. V. Harispe, *Union pharm.* **1939**, 122.

and filter rapidly. Wash the copper sponge thoroughly with hot water. Add about 2 grams of sodium hypophosphite and 100 ml. of concentrated hydrochloric acid to the filtrate. Boil for 15 minutes to precipitate arsenic. A large amount of arsenic may require further addition of sodium hypophosphite and additional boiling. Cool, add 10 ml. of benzene to coagulate colloidal arsenic, shake, and filter through a wet filter. Wash the contents of the filter once or twice with hot water. Heat the filtrate to boiling. If more arsenic separates, add more sodium hypophosphite and repeat the treatment.

Clean a strip of copper foil about 15 by 1.5 cm., coiled in a flat spiral, by warming in 1:1 nitric acid, wash with water, and drop into the boiling solution containing antimony. Boil from 1.5 to 2 hours to plate out the antimony. Pour off the solution and wash the strip quickly with cold water. Unless this washing is completed in a matter of seconds loss of antimony will occur due to oxidation by dissolved oxygen and solution by the wash water. Place the strip in a small beaker and cover with water. At once add about 1 gram of sodium peroxide, and warm until the deposit is dissolved. The steps from washing through addition of the peroxide must be done quickly or the antimony deposit may become insoluble. Pour off the solution, and rinse the copper coil with cold water. The strip will be stained with cupric oxide. Immerse in 1:1 hydrochloric acid to dissolve this oxide. Any residual stains are antimony. If any remain, repeat the treatment with sodium peroxide.

Precipitate copper sulfide from the alkaline solution with hydrogen sulfide, warm about 15 minutes to coagulate, and filter. Wash with 1 per cent ammonium nitrate solution, then discard the precipitate. To the filtrate and washings, add 5 ml. of concentrated sulfuric acid and evaporate to fumes, adding a few drops of concentrated nitric acid near the end of the evaporation. Cool and dissolve in about 15 ml. of water. Use the resulting clear solution in 1:3 sulfuric acid, or an aliquot.

Moderate Amounts of Tin Present.[3] Dissolve a 5-gram sample in about 60 ml. of 1:1 nitric acid and 10 ml. of concentrated sulfuric acid. Evaporate to sulfur trioxide fumes, let cool, and dissolve in cold water. Add about 5 grams of potassium bitartrate and then 14 grams of sodium hypophosphite. Cover and heat. Proceed as in the previous method starting at "When almost to boiling, remove and filter rapidly."

Large Amounts of Tin Present.[4] For this method to be applicable

[3] *Ibid.*

[4] Albert C. Holler, *Anal. Chem.* **19**, 353-5 (1947).

the amount of tin must be at least 10 times the amount of antimony. More tin may be added. Heat a 5-gram sample with 25 ml. of concentrated nitric acid until solution is complete and oxides of nitrogen have been driven off. Add 100 ml. of hot water and digest just below boiling on a hot plate for at least an hour. Antimony is coprecipitated with metastannic acid. Filter and wash the precipitate with hot water. Discard the filtrate. Heat the precipitate and paper with 25 ml. of concentrated nitric acid and 10 ml. of concentrated sulfuric acid and evaporate to sulfur trioxide fumes. If carbonaceous residues persist, add more nitric acid and again take down to fumes. When cool, wash down the sides with 10 ml. of water and again evaporate to fumes. Cool and dilute to about 150 ml. Add 10 ml. of concentrated hydrochloric acid and boil for 2-3 minutes to clear the solution. Cool and dilute to 250 ml. in a volumetric flask. Use an aliquot, usually 25 ml., for development of color with potassium iodide-sodium hypophosphite solution.

Alloys. If 0.05 per cent of antimony is present, take 1 gram of sample; if less, take 5 grams. Dissolve in 50 ml. of concentrated hydrochloric acid with addition of excess bromine in small amounts, while solution is taking place. Add 10 grams of oxalic acid and 350 ml. of water. Boil gently and add about 0.5 gram of sodium hypophosphite to reduce free bromine and render the solution colorless. Revert to the first method for copper-base alloys starting at "Clean a strip of copper foil. . . ."

Lead. Dissolve 20 grams of sample in 100 ml. of hot 1:3 nitric acid. Add water as necessary to dissolve the lead nitrate formed. When a clear solution is obtained stir in 80 ml. of 1:3 sulfuric acid. The lead is precipitated as the sulfate. Chill and filter, washing the filter with cold 1:10 sulfuric acid. Evaporate the filtrate and washings to copious sulfur trioxide fumes. Let cool and take up with 150 ml. of 1:2 hydrochloric acid. Add 5 grams of sodium hypophosphite and boil for 15 minutes. Let it cool, add 20 ml. of benzene, and shake. Filter on a wet paper, so that the benzene will be retained, and wash the residue on the paper with hot water. If there is a generous precipitate of arsenic at this point, repeat, starting with "Add 5 grams of sodium hypophosphite. . . ."

Revert to the first method for copper-base alloys, starting at "Clean a strip of copper foil. . . ."

Refined Lead.[5] Prepare an iron crucible for this determination as follows. Wash the crucible free from rust with concentrated hydrochloric acid and rinse with water. Prepare 1:20 sulfuric acid containing 1 ml. of concentrated nitric acid for each 100 ml. Immerse the crucible in this for 5-6 minutes. Remove, rinse thoroughly with water, and dry with a towel. Then heat in an electric furnace at 450-500° until a dark blue film of magnetite is formed. This usually requires 5-7 minutes and the formation of this film prevents contamination of the melt with iron oxide. Similarly prepare an iron stirrer.

Melt 4 grams of potassium hydroxide and 6 grams of sodium hydroxide in the prepared crucible. Add 100 grams of lead, heat to 350°, and stir for 10 minutes at that temperature. Remove from the heat and let stand for 2-3 minutes until the lead has solidified but the alkaline melt is still liquid. Pour the alkaline melt into a dry porcelain dish and wash the crucible, stirrer, and lead residue several times with 4 per cent sodium hydroxide solution. If the sample did not contain more than 0.01 per cent of antimony, this together with arsenic, tin, and zinc will have been oxidized and be present in the alkaline solution. If somewhat more antimony is present make a second fusion of the same lead sample. Dilute the washings to 100 ml. with 4 per cent sodium hydroxide solution. The alkali avoids hydrolysis of sodium antimonate, sodium arsenate, etc.

Mix a 10 ml. aliquot with 20 ml. of 1:1 sulfuric acid and 1 ml. of fresh 2 per cent sodium sulfite solution. Boil this mixture for 1-2 minutes and cool. Filter off the lead sulfate and wash the filter with 1:3 sulfuric acid. Use this entire solution as sample and consider such sample as being dissolved in 1:3 sulfuric acid in development of color.

Mercury Ores.[6] Heat a sample of 2 grams with 20 ml. of concentrated sulfuric acid until a clear supernatant liquid is obtained. Let cool and take up in about 100 ml. of water. Add 2 grams of tartaric acid, transfer to a 200-ml. volumetric flask with water, and dilute to volume. Filter a portion for use as sample. The ore may contain up to 8 per cent of mercury, 10 per cent of iron, and 5 per cent of arsenic. In development of color allow for the solution as being a 1:10 sulfuric acid solution and determine by potassium iodide and pyridine.

Organic Matter.[7] Add magnesium oxide to the sample in a silica

[5] S. Yu Fainberg, *Zavodskaya Lab.* 6, 36-40 (1937).
[6] M. Ya. Shapiro, *ibid.* 8, 986-8 (1939).
[7] Frank Bamford, *Analyst* 59, 101-2 (1934).

dish until its reaction is basic. Cover with a saturated solution of magnesium nitrate at the rate of 35-40 ml. per 100 grams of sample. Heat on a sand bath with stirring until the charred mass has begun to whiten. Crush with a pestle and continue heating until all the carbon has been consumed. If difficulty is encountered, let the ash cool, moisten with 5 ml. of saturated ammonium nitrate solution, and again heat. Moisten the carbon-free ash with 5 ml. of water and add 1:1 hydrochloric acid until just acid to litmus. Dilute to about 20 ml. and saturate with hydrogen sulfide to precipitate the antimony. Filter and wash.

Dissolve the antimony sulfide from the filter with 2 ml. of concentrated hydrochloric acid. Wash the filter well and add 5 ml. of concentrated sulfuric acid to the filtrate. Heat to copious sulfur trioxide fumes and allow to cool. Dilute to 50 ml. and consider the solution as being in 1:10 sulfuric acid.

Urine.[8] Mix a 25-ml. sample with 4.2 ml. of concentrated sulfuric acid and 10 ml. of concentrated nitric acid in a 100-ml. Kjeldahl flask. Boil gently and add more concentrated nitric acid from time to time to avoid carbonization. Thus possible reduction to trivalent antimony is avoided. When decomposition is complete, cool, add 50 ml. of water, and evaporate it. If any yellow color is now shown, the digestion is incomplete. In that case add a few drops of 30 per cent hydrogen peroxide and heat again for a minute. Dilute to 25 ml. for the use of aliquots. The sample will be in approximately 1:5 sulfuric acid. Determine by the iodide method.

Tissue. Use a sample expected to contain about 0.25 mg. of antimony. Add 4.2 ml. of concentrated sulfuric acid to it in a 100-ml. Kjeldahl flask. Prepare a mixture of 3 volumes of 70 per cent perchloric acid with 1 volume of concentrated nitric acid. Add 10 ml. of this to the flask and boil gently. From time to time add more of the oxidizing mixture in order to avoid carbonization. Complete as for urine starting at "When decomposition is complete. . . ."

Feces. Follow the technic for urine using 0.5 gram of dry or 2 grams of wet sample.

Plasma. The preparation of sample has been shown under bismuth (page 160). Take an aliquot for determination by the iodide method.

[8] Evan W. McChesney, *Ind. Eng Chem*, *Anal. Ed* 18, 146 9 (1946).

The method for determination of bismuth by the iodide method (page 164) includes the determination of antimony.

Biological Samples.[9] Transfer about 15 grams of blood or tissue, or 50 ml. of plasma or urine, to a flask. Add 5 ml. of concentrated nitric acid, glass beads, and 1 ml. of capryl alcohol. The digestion will begin spontaneously and is facilitated by occasional shaking. When the initial reaction ceases place on a hot plate at low heat. When nitric acid fumes are evolved, advance the heat. If charring appears, remove from the heat, let cool, and add 2 ml. of concentrated nitric acid. Mix well and reheat. Repeat as necessary. Finally heat to a clear yellow or colorless digestate. Add 2 drops of 60 per cent perchloric acid and heat to sulfur trioxide fumes. The cooled solution should be water white. Add 3 ml. of water and heat to sulfur trioxide fumes. This is the sample for development of color by the Rhodamine B method.

Separation from Arsenic and Tin.[10] The separation of arsenic by distillation from strong hydrochloric acid solution has been described (page 183). Antimony and tin remain in the flask. To determine antimony add 7 ml. of 85 per cent orthophosphoric acid to the distilling flask and heat to 155-165°. Use 50 ml. of water in the receiver and add 100 ml. of concentrated hydrochloric acid dropwise to the distilling liquid. Use the distillate as the antimony sample. Tin remains in the distilling flask.

Standard

Dissolve 0.1000 gram of antimony in 25 ml. of hot concentrated sulfuric acid. Let it cool and dilute to 100 ml. with water. Make up to 1 liter with 1:3 sulfuric acid. *Each ml. corresponds to 0.1 mg. of antimony.* For a more dilute standard, pipet 10 ml. into a 100-ml. flask and make up to volume with 1:3 sulfuric acid. This contains 0.01 mg. of antimony per ml. The standards are in 1:3 sulfuric acid.

Antimony by Rhodamine B

Pentavalent antimony reacts with Rhodamine B in mineral acid solution in the presence of chloride ion to give a red compound, insoluble

[9] Thomas H. Maren, *Anal. Chem.* **19,** 487-91 (1947).
[10] W. Heuss, *Rev. tech. Sulzer* **1945,** 110-14.

below 10°.[11] Earlier technics involved destruction of excess dye by addition of a moderate excess of bromine and prompt removal of the bromine, a critical operation. For colorimetric estimation, the complex was then dissolved by addition of alcohol. A carefully determined blank was essential and the method requires experience in its manipulation.

Further work[12] has led to technics in which the color is developed in the presence of benzene and immediately extracted or, when interfering amounts of iron are present, the antimony extracted into isopropyl ether and the color developed there.

The amount of sulfuric acid in the sample developed can affect the color and must be constant in samples and standards. Nitric acid will not convert tetravalent antimony to pentavalent, but perchloric acid will. Photometric results at 565 mμ[13] are satisfactory.

Arsenic shows interference at 1 mg., copper and ferric iron at 10 mg. There is no interference by bismuth, zinc, tin, lead, mercury, molybdenum, tungsten, or cobalt.

Procedure. The sample should be available in less than 5 ml. of concentrated sulfuric acid. For many preparations of sample this simply means to stop before the final dilution. Cool the flask so that the operations can be conducted at under 23°. Add 5 ml. of 1:1 hydrochloric acid A deep yellow color at this stage is due to iron and the isopropyl ether technic must be used. Otherwise use the benzene extraction.

Benzene Extraction. Add 8 ml. of orthophosphoric acid, prepared by dilution of 7 ml. of the 85 per cent acid to 100 ml., and 5 ml. of 0 02 per cent Rhodamine B solution. Mix well and pour into a separatory funnel. Delay at this stage is not permissible. Shake 150 times and separate the colored benzene layer. Read at 565 mμ or with a green filter. This color is stable for 4 hours. If the color is too intense, dilute with more solvent. Iron will give a positive blank.

Isopropyl Ether Extraction. Add 13 ml. of water and transfer to a separatory funnel. Rinse the flask with 15 ml. of isopropyl ether and

[11] E. Eegriwe, *Z. anal. Chem.* 70, 400-3 (1927); William G. Frederick, *Ind. Eng. Chem., Anal. Ed.* 13, 922-4 (1941); A. Hassan, *J. Roy. Egypt. Med. Assoc.* 25, 307-20 (1942); Stewart H. Webster and Lawrence T. Fairhall, *J. Ind. Hyg. Toxicol.* 27, 183-92 (1945).

[12] Thomas H. Maren, *Bull. Johns Hopkins Hospital* 77, 338 (1945); *Anal. Chem.* 19, 487-91 (1947); Leon D. Freedman, *ibid.* 19, 502 (1947).

[13] A. Gellhorn, M E. Krahl and J. W. Fertig, *J. Pharmacol* 87, 159 (1946).

To one tube add the aliquot of sample which must not contain over 1 mg. of antimony, usually 10 ml. or 20 ml. To the other add a volume of standard containing the amount of antimony expected in the sample. To the sample tube add a volume of 1:3 sulfuric acid equal to the amount of standard added to the other. To the standard tube add the same concentration of sulfuric acid as is present in the sample, and the same volume. Thus the acidity of sample and standard is uniform. Dilute each to volume with 1:3 sulfuric acid, mix, and compare. If the sample solution develops turbidity, dilute with an equal volume of the mixed reagents, diluted to 100 ml. with water.

If the color developed is low due to limited antimony content, and a larger aliquot cannot be used, extract the solutions of sample and standard developed for comparison of color, with 10 ml. of amyl alcohol, then with 5 ml. Combine the extracts from the sample and from the standard and compare by balancing.

Antimony as the Sulfide

Antimony in trivalent form may be determined as the orange colloidal sulfide.[18] Bismuth does not interfere. Accuracy to 5 per cent is usually attainable by matching the concentrations of salts in sample and standard.

Procedure. Neutralize an aliquot of the sample containing 0.02-0.2 mg. of antimony with 1:1 hydrochloric acid, or with 10 per cent sodium hydroxide solution, then make just acid with 1:1 hydrochloric acid. Take a suitable aliquot of standard antimony solution and, depending on the previous history of the sample, add the same acid or alkali and similarly neutralize. Dilute each to about 60 ml. and add 20 ml. of saturated aqueous sulfur dioxide solution. Boil until evaporated to about 10 ml. This reduces all pentavalent antimony to the trivalent form.

Let cool and add 5 ml. of a 1 per cent solution of gum arabic to each. This will stabilize the colloidal sulfide when developed. Transfer to Nessler tubes, add 10 ml. of concentrated hydrochloric acid, and dilute to about 70 ml. To each add 20 ml. of a freshly saturated aqueous solution of hydrogen sulfide, dilute to volume, mix, and compare by dilution.

Antimony by Molybdenum Blue

This familiar reaction has been applied to the determination of

[18] B. S. Evans, *Analyst* 47, 1-9 (1922).

antimony.[19] The action is that of antimonous ion on phosphomolybdic acid. The presence of iron will increase the intensity of color developed. Stannic ion does not interfere unless it is more than double the amount of antimony. Cupric ion should not exceed 1 mg. in the sample taken.

Procedure. Prepare a fresh phosphomolybdic-acid reagent as follows. Dissolve 20.6 grams of anhydrous sodium molybdate in 100 ml. of water. Add 3 grams of dibasic sodium phosphate dodecahydrate dissolved in 25 ml. of water by heating. Add 1:1 nitric acid dropwise until the solution turns to a golden yellow and is approximately at pH 3.0.

Transfer a sample containing 0.05-0.5 mg. of antimony, neutralize if necessary, and dilute to 25 ml. Add 5 ml. of 1:4 sulfuric acid. Add 3 ml. of saturated sulfurous acid and boil until all the sulfur dioxide has been driven off. Again dilute to 30 ml. Add 1 ml. of the phosphomolybdic acid reagent and heat for 10 minutes on a boiling water bath. Cool to room temperature and add 8 ml. of 1:4 sulfuric acid to decompose excess reagent. Shake it periodically for 5 minutes, transfer to a 50-ml. volumetric flask, and dilute to volume. Read the blue color with the photometer and compare with a calibration curve.

Miscellaneous

The reaction of trivalent antimony with phosphomolybdotungstic acid may be used for its colorimetric estimation.[20] The method will determine 0.05 mg of antimony in 100 ml. of solution Prepare the sample and standard as for determination as the sulfide through "This reduces all pentavalent antimony to the trivalent form." To each add 5 ml. of 1:10 hydrochloric acid, or alternatively make just acid to β-dinitrophenol as indicator. Add Folin's reagent (page 623), phosphomolybdotungstic acid, acidified to less than 0 1 N, until a suitable color is developed. The amount of reagent required varies with the antimony content. Heat the solution to develop the full color. Compare the sample and standard.

In the absence of arsenic, the antimony may be evolved as stibine and determined by the stain on mercuric chloride paper, as in the Gutzeit method for arsenic.[21]

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[19] A. I. Kokorin, *Zavodskaya Lab.* **12**, 64 8 (1946).

[20] Shoji Makishima, *J. Soc. Chem. Ind. Japan* **34**, Suppl. Binding 322-3 (1931).

[21] Jehiel Davidson, George N Pulley, and C. C. Cassil, *J. Assoc. Official Agr. Chem.* **21**, 314-18 (1938).

an aliquot of this. Alternatively use the solution as prepared for copper determination (page 95).

If it is necessary to start with a new sample solely for tin,[5] evaporate 100-200 ml. in a silica dish and ignite in a muffle at about 550° to a white fluffy ash. Too high a temperature will result in fusion. Transfer the ash to a 00 high-form porcelain crucible. Quickly grind 1 part of potassium cyanide with 3 parts of anhydrous sodium carbonate to give a homogeneous powder. Cover the ash with 1 gram of this fusion mixture and fuse over a Meker burner for 15 seconds, swirling during the operation by holding the crucible with tongs. Do not overheat or fuse too long. Let cool and place in a covered beaker under a hood to take care of hydrocyanic acid evolved from here on. Pipet 5 ml. of 1:1 hydrochloric acid into the crucible in the covered beaker. After reaction ceases wash down the cover glass into the crucible. Overturn the crucible with a glass rod and heat the beaker. Further gas evolution usually occurs. When gassing is over, remove the crucible with a stirring rod and wash well. Boil down the solution and washings in the covered beaker to less than 10 ml. Filter this ash solution into a flask and wash the residue with hot water. This has been found a satisfactory sample for determination as the sulfide.

Biological Samples.[6] Wet ash (page 178) and transfer the solution to the distillation equipment as described for ferrous metals (page 212). Add 15 ml. of concentrated sulfuric acid and complete, starting at "Add glass heads to minimize . . ."

Foodstuffs. A filtrate from sulfide separation of copper and lead is provided under copper (page 98). Boil off the hydrogen sulfide and, if necessary, filter to remove coagulated sulfur. Dilute to a known volume for the use of aliquots.

If it is necessary to start off with an original sample,[7] digest 10 grams with 10 grams of potassium sulfate and 30 ml. of concentrated sulfuric acid until all organic matter is destroyed, adding more acid if necessary. When cool, dilute to about 100 ml. and neutralize to litmus by addition of concentrated ammonium hydroxide. Cool and pass in hydrogen sulfide for 10 minutes, then put under hydrogen sulfide pressure for an hour. Filter until the filtrate is colorless. Repeat the treatment of the

[5] Irwin Stone, *Ind. Eng. Chem., Anal. Ed.* 13, 791-2 (1941).
[6] J. Schwaibold, W. Borchers and G. Nagel, *Biochem. Z.* 306, 113-22 (1940).
[7] R. DeGiacomi, *Analyst* 65, 216-18 (1940).

filtrate with hydrogen sulfide and if any further precipitate is obtained, filter.

Digest the precipitate and paper on a steam bath with 10 ml. of 10 per cent sodium hydroxide solution. Filter and wash the filter. Make this solution acid with 1:1 hydrochloric acid and use all or an aliquot for development of color.

Alternatively,[8] incinerate 5-20 grams in a silica dish and transfer the ash to the distillation equipment described for ferrous metals (page 212). Digest any residue on the dish with 10 ml. of concentrated sulfuric acid. Add more concentrated sulfuric acid and continue as for ferrous metals (page 212) starting at "Add glass beads to minimize bumping . . ."

Separation by Distillation. The separation of arsenic (page 183) and antimony (page 206) by distillation has been described. Heat the solution to 140-145° and add dropwise, while maintaining the temperature, a mixture of 1 part of concentrated hydrobromic acid and 3 parts of concentrated hydrochloric acid. Absorb the distillate in 100 ml. of water and continue the distillation according to the tin content. Use all or an aliquot as sample and, if tin is to be present as stannous chloride, add aluminum wire to reduce the stannic tin.

Separation of Copper, Zinc, Bismuth, Lead, and Tin. A method of separation is shown under lead (page 33). The filtrate as separated is ready for use as sample solution for aliquots.

STANDARD

To prepare the standard solution, dissolve 1 gram of pure tin in 100 ml. of 1:1 hydrochloric acid and dilute to 1 liter with the same acid. This standard contains 1 mg. of tin per ml. For preparation of a standard containing 0.1 mg. per ml. dilute 10 ml. to 100 ml. with 1:1 hydrochloric acid and for a standard containing 0.01 mg. per liter dilute 5 ml. to 500 ml. with the same acid.

TIN BY MOLYBDENUM BLUE

Just as phosphate in the presence of excess molybdate and reducing agent such as stannous chloride give molybdenum blue as a measurement of phosphate, or under other conditions a measure of molybdenum,

[8] N. H. Law, *Analyst* 67, 283-7 (1942).

so by varying the conditions this reaction measures the tin present as stannous chloride.[9] To obtain a standard composition of the resulting blue it is necessary to standardize conditions rigidly. A large excess of molybdate reagent is necessary to develop the maximum intensity. Not over 0.03 mg. of tin per ml. is permissible. The color develops over a period of 30 minutes and after reaching its maximum intensity does not fade for at least 90 minutes.

As little as 0.1 mg. of tin can be determined. Oxidizing agents and other reducing agents must be absent. Antimony gives a faint greenish color which will not be confused with the blue of tin but may make readings difficult. Arsenic and zinc do not interfere. Copper interferes but the tin can be separated from it by precipitation as the sulfide, the copper being retained as a thiourea complex or as the complex cyanide. Titanium interferes but can be retained in solution by tartaric acid when precipitating tin as the sulfide. For greater sensitivity the blue color developed can be extracted with amyl alcohol.

The corresponding reaction as the silicomolybdate gives an equivalent color and has advantages.[10] The silica complex $H_4[Si(Mo_3O_{10})_4] \cdot nH_2O$ is more sensitive to reducing agents and more stable. In the phosphomolybdate procedure, at very high acidity the blue color is not formed, at lower acidities inconsistent results were obtained and fading occurred. The presence of silica in the phosphomolybdate reagent, as from that in the alkali used, will give a yellow reagent. Deviations in the blue color are best avoided by reducing the tin with zinc shot, adding the reagent to this while still being reduced, and decanting at once.

For quantities of 0.02-0.2 mg. of tin by the micromethod the accuracy is within 15 per cent and for 0.2-1 mg. by the macro method accuracy is within 5 per cent. Comparison by balancing or by transmittance gives similar accuracy. The final acidity may vary over the range of 0.82-1.72 *N* with little effect on the color developed. The macro method shows little interference up to 5 mg. of arsenic or 3 mg. of antimony. Over 0.2 mg. of iron lessens the color and gives a greenish cast. Permanent artificial standards are feasible, just as for the phosphate determination, Vol. 1, p. 55.

Procedure. Prepare a reagent as follows. Fuse 1 gram of pure silica with 5 grams of sodium carbonate, dissolve in water, and dilute

[9] Longstaff, *Chem. News* 80, 282 (1899); G. F. Hüttig, *Chem.-Ztg.* 47, 341-2 (1923); N. Strafford, *Mikrochem. Acta* 2, 306-13 (1937).

[10] Irvin Baker, Martin Miller and R. Stevens Gibbs, *Ind. Eng. Chem., Anal. Ed.* 16, 269-71 (1944).

to 1 liter. This contains 1 mg. of silica per ml. Dissolve 5.3 grams of ammonium molybdate in about 100 ml. of water, add 10 ml. of concentrated sulfuric acid, and dilute to 200 ml. To prepare the mixed reagent from these daily, dilute 10 ml. of the molybdate solution to about 800 ml., add 2.5 ml. of the silicate solution, and dilute to 1 liter. After mixing thoroughly, let this stand for 0.5 hour before use. After 24 hours some decomposition of the heteropoly complex occurs.

Macro. For 0.2-1.0 mg. evaporate the sample to under 25 ml. The treatment depends on the past history of the sample but finally as diluted to 30 ml. it should be 1:1 hydrochloric acid. Select a suitable volume of standard to contain the same salts, acid, etc., in the same volume. Heat each to boiling and add about 5 grams of zinc shot of reagent grade, low in arsenic, lead, and iron. Agitate while boiling for 1 minute. Add 100 ml. of the reagent to the flasks containing sample and standard, mix, and decant within 10 seconds into stoppered storage flasks. Compare the colors after 5 minutes. No color is given by a blank if the separation is carried out within 10 seconds. There is appreciable fading in 30 minutes at some concentrations.

Micro. For 0.02-0.2 mg. of tin follow the macro procedure with these changes. The final volume of sample is 6 ml. Add about 1 gram of zinc shot. Use 20 ml. of reagent.

Extraction. If greater sensitivity is necessary extract the blue color from sample and standard with 10 ml. of amyl alcohol and compare.

Tin by Dithiol

The reagent, 1-methyl-3,4-dimercaptobenzene, is known as dithiol and gives a red precipitate with tin which may be dispersed and used for colorimetric estimation.[11] The reaction occurs more quickly with stannous ion than with stannic. A reagent in which the methyl group is replaced by chlorine gives a similar result. The usual range of determination is 1.5-6 ppm but the method has been found satisfactory for determining 0.1 ppm. of tin in beer, an amount which causes clouding.

Numerous metals give precipitates with the reagent but that with tin is characteristic. Bismuth gives a different shade of red. Excessive iron

[11] R. E. D. Clark, *Analyst* 61, 242-5 (1936); *ibid.* 62, 661-3 (1937); J. Hubert Hamence, *ibid.* 62, 18-23 (1937); Irwin Stone, *Ind. Eng. Chem., Anal. Ed.* 13, 791-2 (1941).

may be removed from a sample as Prussian blue. Hydrogen sulfide in the sample solution is not objectionable. Acidity of sample and standard must be closely matched.

In the presence of 0.5 ppm. of tin there was no interference by 10 ppm. of iron or lead. Manganese, nickel, and zinc caused low results; bismuth, cobalt, and copper negative results. Any of these metals as oxides could be acid-extracted from ash, leaving the insoluble tin oxide. The use of thioglycolic acid as solvent for the reagent insures reduction of tin. The Lovibond tintometer has been used as a permanent color record.

The reagent may be solubilized with sodium hydroxide, in which case deterioration will show as a white precipitate or disulfide. When added to solutions containing up to 1:6 hydrochloric acid the colors developed are as follows. One ppm. of stannous ion gives an immediate pink color, time must be allowed for reduction of stannic ion unless thioglycolic acid is present. Silver gives yellow; mercury, cadmium, or arsenic a pale yellow; copper, cobalt, or nickel black; bismuth brick red; lead bright yellow. A protective colloid is required if the tin is greater than 30 ppm. Thioglycolic acid in the reagent increases its stability, as well as serving as a reducing agent.

Procedure. Add 0.25 ml. of melted dithiol to 10 ml. of thioglycolic acid and dissolve. Dilute to 200 ml. with 95 per cent ethanol and store in small tightly-stoppered bottles in the dark. It is stable if protected from the air but becomes oxidized and ineffective on exposure.

Transfer a 10-ml. sample to a test tube and suitable amounts of standards adjusted to the composition of the sample to other tubes. Neutralize each to litmus with concentrated ammonium hydroxide, and add 0.5 ml. of concentrated hydrochloric acid. Add 0.5 ml. of the reagent to each sample and standard, mix, and heat in a boiling water bath for 1 minute. Cool and add 2 ml. of a 10 per cent solution of gum arabic, preserved with 100 ppm. of phenylmercuric acetate. Shake and compare the reflection from the resulting turbidities. The colors are stable for at least a month if kept sealed and in the dark. They must be shaken immediately before comparing.

Tin as the Sulfide

As another of the sulfide methods, tin may be so determined in the absence of interfering ions.[12] The comparisons are of colloidal sulfides.

[12] Rudolf Hanssen, *Chem. Ztg.* 54, 143 (1930); Gerald M. Compeau and Eugene W. Blank, *Oil and Soap* 21, 275-6 (1944).

Stannous sulfide is unsuitable for the purpose but by suitable manipulation both stannous and stannic ions may be estimated. Since it is necessary to make a preliminary oxidation with bromine and some excess remains, this oxidizes some hydrogen sulfide to free sulfur. The error so introduced is not great but does tend toward high results.[13] This criticism does not apply to the solutions where bromine has not been added. Amounts of 1-5 mg. per 100 ml. can be satisfactorily estimated by this method.

Procedure. *Total Tin.* Select a portion of sample for the purpose, such as 10 ml., and parallel it with a suitable series of standards. Add corresponding salts and acids to the standards according to the previous history of the sample. Approximately neutralize the sample and standards with 40 per cent sodium hydroxide solution. For each 10 ml. of sample or standard add 0.4 ml. of concentrated hydrochloric acid and a drop of saturated bromine water, more bromine water if that does not give a faint trace of excess when a drop is put on potassium-iodide-starch paper. All the stannous ion has now been oxidized to stannic ion. Mix each with half its volume of a saturated solution of hydrogen sulfide and allow to stand for 1 hour to complete formation of the colloidal sulfide. Shake well to resuspend the sulfide for comparison.

Stannic Tin. In a sample solution where the ratio of stannous to stannic ion has not been affected by the method of preparation of sample, the amount in each form can be determined. Mix a portion of sample with an equal volume of saturated aqueous solution of hydrogen sulfide, and let stand for 1 hour without undue exposure to air. Add a volume of concentrated hydrochloric acid equal to the volume of original sample taken. The stannous sulfide goes into solution at once and the effect of the acid on the stannic sulfide after it has been completely formed is so slight that it may be neglected. Compare the color with that of a series of known stannic sulfide suspensions.

Stannous Tin. Subtract the stannic tin from the total tin, after allowing for the differences in aliquots used, to give the stannous tin content.

Miscellaneous

Stannous compounds produce a characteristic amethyst coloration with cacotheline which has been used as a method of colorimetric esti-

[13] Irvin Baker, Martin Miller and R. Stevens Gibbs, *Ind. Eng. Chem., Anal. Ed.* 16, 269 71 (1944).

mation,[14] although adversely criticized. The reagent, which is a nitrobrucine of unknown structure, will detect 1 ppm. of tin in 1:10 hydrochloric acid in about 15 minutes, or 2 ppm. in water in about 45 minutes. Nitric acid prevents the reaction and sulfuric acid lowers the sensitivity. The color fades after a few hours. Substantial amounts of antimony also give a color with the reagent. Bisulfite, hydrosulfite, sulfite, titanous, mercuric, chromate, chromic, and nitrate ions interfere. Ions such as molybdate, which react with the tin, must also be absent. Colored ions such as cobalt, copper, iron, nickel, and vanadium must be low.

First prepare a reagent as follows: Dissolve 39.4 grams of dry brucine in 200 ml. of 1:3 nitric acid in the cold, then warm to 50-60° for 15 minutes. The red solution becomes reddish yellow and crystals of cacotheline separate out. Let stand in ice water for a few hours to complete the crystallization. Filter by suction and wash the crystalline material with 1:20 nitric acid, acetone, and ether. The yield should be 86-89 per cent of theoretical, or about 44-45 grams. From this prepare a 0.25 per cent solution. To 10 ml. of sample solution of about 1:10 acidity with hydrochloric acid and a standard similarly adjusted, add 0.1 ml. of reagent solution. Mix and let stand until the color develops to a maximum. Compare by balancing, or dilution with 1:10 hydrochloric acid.

Other reagents used are α-dinitrodiphenylamine sulfoxide,[15] dimethylglyoxime,[16] hematoxylin,[17] and quinalizarin.[18]

Tin forms a stannous dithizonate extractable with chloroform over the pH range 3-9.[19] Although not developed as a colorimetric method, the reaction shows promise.

[14] Hermann Leuchs and Friedrich Leuchs, *Ber.* 43, 1042-51 (1910); Hermann Leuchs and Hans Kaehrn, *ibid.* 55, 724-32 (1922); Hermann Leuchs, Bernhard Winkler and W. Robert Leuchs, *ibid.* 55, 3936-50 (1922); Hermann Leuchs, Fritz Osterburg and Hans Kaehrn, *ibid.* 55, 564-72 (1922); Gregoire Gutzeit, *Helv. Chim. Acta* 12, 720 (1929); I. Laird Newell, Joseph B. Ficklen and Lewis S. Maxfield, *Ind. Eng. Chem., Anal. Ed.* 7, 26-7 (1935); Irvin Baker, Martin Miller and R. Stevens Gibbs, *ibid.* 16, 269-71 (1944).

[15] G. S. Buchanan and S. B. Schryver, *Analyst* 34, 121 (1909).

[16] L. Tschugaeff and B. Orelkin, *Z. anorg. Chem.* 89, 401-4 (1914).

[17] V. Ya. Tartakovskii, *Zavodskaya Lab.* 9, 971-5 (1940).

[18] V. I. Kuznetsov and I. M. Bender, *J. Applied Chem.* (U.S.S.R.) 13, 1724-31 (1940); V. I. Kuznetsov, *ibid.* 13, 769-75 (1940).

[19] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66-72 (1939).

CHAPTER 11

INDIUM

INDIUM OCCURS in association with zinc but is relatively unimportant. Its commercial importance has increased markedly in the last few years and efforts have been made to find rapid and accurate methods for its determination.

SAMPLES

Alloys. Weigh out a sample which will contain 10-15 mg. of indium. Initially dissolve in a mixture of 3 parts of concentrated hydrochloric acid and 1 part of concentrated nitric acid. Let cool and add 5 ml. of concentrated sulfuric acid. Heat to fumes of sulfur trioxide and let cool. Add about 5 ml. of water and again take to sulfur trioxide fumes to destroy nitrosyl sulfuric acid, and let cool. Dilute to approximately 200 ml., and add 3 ml. of concentrated hydrochloric acid. This solution is intended to be approximately N to prevent precipitation of indium sulfide at the next step.

Heat to boiling on a hot plate and pass hydrogen sulfide through the boiling solution for 30 minutes to precipitate silver, mercury, lead, and other sulfides insoluble in N acid. These sulfides have been stated to carry down some indium by sorption.[1] Filter the hot solution at once and boil the filtrate until free from hydrogen sulfide. Let cool and add 1:1 ammonium hydroxide until slight excess is shown by odor. Digest on a steam bath until the precipitate of indium hydroxide is no longer gelatinous and filter at once. Wash the filter with a minimum of hot water and discard the filtrate and washings.

Dissolve the precipitate from the filter with 15 ml. of warm glacial acetic acid, poured through several times, and finally use a 100-ml. volumetric flask as receiver. Wash the filter with 10 ml. of warm glacial acetic acid, then 3 times with 5-ml. portions of hot water. Dilute the filtrate and washings to volume as a sample containing 0.1-0.15 mg. of indium per ml.

[1] E. B. Sandell, "Colorimetric Determination of Traces of Metals," p. 257. Interscience Publishers, Inc., New York, N. Y. (1944).

Standard

Digest a 5-gram sample of indium that is 99.98 per cent pure[2] with hot 1:7 sulfuric acid until only a small amount of metal remains undissolved.[3] Add 1:15 ammonium hydroxide to the filtered solution to precipitate indium hydroxide. Filter and wash the residue with a 1:50 ammonium hydroxide solution. Dissolve the residue by digesting with 10 ml. of 1:7 sulfuric acid. Repeat the precipitation and solution of indium hydroxide two additional times. Add 10 ml. more of 1:7 sulfuric acid,[4] and heat to about 50°. The acid indium sulfate will crystallize from solution. Wash the crystals with 6 per cent acetic acid and dry. Heat the crystals in a muffle furnace to 250°, cool, and transfer to a tightly-covered bottle.

Weigh 0.226 gram of the prepared anhydrous indium sulfate, dissolve in water, and dilute to 1 liter. This contains 0.1 mg. of indium per ml. If a greater dilution is desired, dilute 10 ml. of this solution to 100 ml. to give 0.01 mg. per ml.

Indium by 8-Hydroxyquinoline

The 8-hydroxyquinoline derivative of indium can be extracted with chloroform, yielding a bright yellow solution that exhibits a fairly broad absorption band at 400 mμ. At this wave length, these solutions obey Beer's law up to concentrations of 18.0 mg. per liter. The optimum pH conditions lie within the range 3.2 to 4.5. This method can be used to determine concentrations of indium ranging from 0.3 to 20.0 mg. per liter of chloroform.[5] Aluminum, gallium, thallium, stannous tin, bismuth, cupric copper, ferric iron, vanadium, molybdenum, nickel, and cobalt, which can also be extracted under the same conditions, interfere with the estimation of indium by this method.

Procedure. Take an aliquot of the sample solution containing about 0.5 mg. of indium. Adjust the pH to approximately 3.2-4.5 by addition of glacial acetic acid or sodium acetate solution. Cool to room temperature and add, with shaking, four 5-ml. portions of 0.15 per cent 8-hydroxyquinoline solution in chloroform, removing the solvent after

[2] Indium Corporation of America, Utica, N. Y.

[3] Therald Moeller, *J. Am. Chem. Soc.* **62**, 2444-6 (1940).

[4] Ralph P. Seward, *J. Am. Chem. Soc.* **55**, 2740-4 (1933).

[5] Therald Moeller, *Ind. Eng. Chem., Anal. Ed.* **15**, 270-2 (1943).

each addition. Combine the resulting extracts and dilute to 50 ml. with chloroform. Read the yellow color, using a filter with maximum transmission around 400 mμ. Compare with a predetermined curve. It is also feasible to estimate the indium content by determination of the fluorescence of this chloroform extract.[6]

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[6] E. B. Sandell, *ibid.* 13, 844-5 (1941).

CHAPTER 12

GALLIUM

Gallium in amounts less than 0.01 per cent is commonly present in iron, aluminum, chromium, and zinc ores. It carries through in the metal refined from those ores. The reactions used for colorimetric estimation are formation of lakes not unlike those for aluminum. It is probable that several methods developed for aluminum would also be applicable.

Samples

Metals. The method depends upon the other metals present, which may have to be removed before proceeding colorimetrically. Dissolve the sample in water or acid according to its nature. Large amounts of alkali metals will interfere. If in acid solution nearly neutralize with 1:1 ammonium hydroxide. Dilute this solution to a known volume for the use of aliquots. There is no satisfactory method for removal of vanadium or molybdenum.

Aluminum Present.[1] If the sample solution on dilution according to the procedure would contain more than 15 ppm. of aluminum, take an aliquot to contain 0.004-0.04 mg. of gallium and dilute to 20 ml. with water. Add 1:1 ammonium hydroxide until turbid. Add 1:1 hydrochloric acid until the solution has cleared and 4 ml. in excess. To this solution, add 19.3 grams of ammonium acetate and 6.7 grams of ammonium chloride. Dilute to 70-80 ml., heat to 75° and add a saturated solution of sodium fluoride, 4.3 grams per 100 ml., slowly with stirring. When the fine crystalline precipitate of sodium aluminum fluoride, Na_3AlF_6, has formed, add an additional 3 ml. of the sodium fluoride solution. Cover and allow to stand for 1.5 hours, stirring every 10 minutes. Add shredded paper pulp, stir, and filter into a 250-ml. volumetric flask. Wash the precipitate with 0.01 per cent sodium fluoride solution, and dilute the filtrate to volume for the use of an aliquot for determination by quinalizarin.

[1] H. H. Willard and H. C. Fogg, *J. Am. Chem. Soc.* 59, 40-5 (1937).

Iron and Indium Present. If the sample solution on dilution according to the procedure would contain more than 1 ppm. of iron or 100 ppm. of indium, take an aliquot to contain 0.01-0.06 mg. of gallium, and dilute to 20 ml. with water. Heat nearly to boiling and slowly add 6 ml. of a 12 per cent sodium hydroxide solution. Heat gently until the ferric and indium hydroxides have coagulated. If the precipitate is indium hydroxide, or if less than 0.2 mg. of iron is present, filtration is unnecessary. Otherwise, add paper pulp predigested with hot 6 per cent sodium hydroxide solution, and washed with water until free of alkali. Stir, filter, and wash the precipitate with hot 1 per cent sodium chloride solution made alkaline with sodium hydroxide.

Heat the filtrate, or the solution with precipitate, nearly to boiling, and add 8-10 drops of a 1 per cent solution of potassium permanganate. Let stand for 2 minutes and add 5-10 drops of 95 per cent ethanol to reduce the solution. Continue heating until all the green color has disappeared and the brown hydrate of manganese dioxide has formed. This serves as a collector for the precipitate. Filter into a 250-ml. volumetic flask and wash the precipitate with hot 1 per cent alkaline sodium chlorido solution. Neutralize the combined washings and filtrate with 1:3 hydrochloric acid until neutral to litmus. Add 19.3 grams of ammonium acetate, 6.7 grams of ammonium chloride, and 3 ml. of saturated sodium fluoride solution. Make up to 250 ml. for the use of an aliquot for determination by quinalizarin.

Zinc and Aluminum or Iron Present. If zinc and aluminum are present, take an aliquot containing 0.01-0.06 mg. of gallium, dilute to 20 ml., and proceed as for the separation of aluminum (page 224), starting at "Add 1:1 ammonium hydroxide until turbid." If zinc and iron are present, take a similar aliquot, dilute to 20 ml., and follow the method for the preparation of a sample containing iron, starting at "Heat nearly to boiling . . ." but add sufficient sodium hydroxide to redissolve the zinc hydroxide.

Iron and Aluminum Present. Separate the iron as described up to the point where the filtrate has been neutralized. Acidify the filtrate with 1:3 hydrochloric acid. Precipitate the aluminum and gallium hydroxides by the conventional method for aluminum hydroxide by ammonium hydroxide, avoiding large excess of the alkali which will redissolve some gallium hydroxide.

Purify anthracene by dissolving in hot acetone and pouring into an equal volume of concentrated hydrochloric acid. Filter, wash with

hot 1:100 hydrochloric acid and with hot water. Recrystallize from a 1:1 mixture of acetone and toluene. To check the purity, extract a portion with 1:10 hydrochloric acid. When nearly neutralized it should give no color with the reagent. Filter the precipitated hydroxides on a Gooch crucible containing a mat of this anthracene. Transfer the precipitate and mat to the original beaker and boil with 1:1 hydrochloric acid until the precipitate is completely dissolved. Wash a filter paper with hot 1:1 hydrochloric acid and filter the boiled solution through it. Separate aluminum, if in excess of 0.5 mg., by sodium fluoride (page 224) starting at "Add 1:1 ammonium hydroxide until turbid" and complete by that method. Otherwise dilute to 250 ml. for the use of aliquots for determination by the quinalizarin method.

Lead, Copper, Tin, Antimony, Germanium or Platinum Present. Take an aliquot containing 0.01-0.06 mg. of gallium. If germanium is present dilute to 20 ml. with concentrated hydrochloric acid, or add an equal volume of concentrated hydrochloric acid, whichever is the greater. Boil until reduced to the original volume, thus volatilizing germanium.

Dilute to 20 ml. and add a small excess of 40-60 mesh cadmium metal to precipitate less active metals. When precipitation is complete, filter, and add 2-3 ml. of concentrated sulfuric acid to the filtrate. Evaporate to fumes of sulfur trioxide, cool, and add 100 ml. of water. Remove cadmium by electrolysis. Saturate the solution with hydrogen sulfide to remove the last traces of cadmium. Filter and wash the precipitate. Reduce the combined filtrate and washings to about 20 ml. Add an excess of 10 per cent sodium hydroxide solution and proceed as described for separation of iron and indium beginning at "Heat the filtrate, or the solution with precipitate, nearly to boiling. . . ."

Minerals.[2] Heat a 0.25-gram sample of 100-mesh material with 2 ml. of 1:5 sulfuric acid and 3 ml. of 48 per cent hydrofluoric acid. Finally drive off sulfur trioxide fumes but avoid decomposition of ferric sulfate. Take up the cooled residue in about 2 ml. of 1:20 sulfuric acid and again fume off excess sulfuric acid. Take up the residue in 10 ml. of 1:1 hydrochloric acid, warming and stirring until solution is complete. After heating for at least a half hour, filter to remove insolubles such as calcium sulfate and wash the residue on the paper with 1:100 hydrochloric acid. Use a 25-ml. volumetric flask as receiver. Add about 0.5 gram of fine silver powder prepared by displacement from silver nitrate

[2] E. B. Sandell, *Anal. Chem.* 19, 63-5 (1947).

by metallic copper. Swirl until the ferric iron is nearly all reduced as shown by substantial disappearance of the yellow color. This usually requires 1-5 minutes. Add 8 ml. of concentrated hydrochloric acid and dilute to volume. Mix for a minute.

Prepare a funnel with a plug of glass wool at the bottom of the stem, filled loosely over that with 15 mm. of powdered silver. This should be such as to pass 5-7 ml. of solution per minute. After use, rinse with 1:1 hydrochloric acid and dry for reuse. Pass a little more than 10 ml. of the solution through this and immediately transfer 10 ml. for extraction to a separatory funnel prerinsed with 1:1 hydrochloric acid.

Add about 8 ml. of ethyl ether to the funnel and shake for half a minute. When the layers separate, withdraw the acid layer into a similar funnel and extract with 5 ml. of ether. Discard the acid layer and combine the ether extracts which contain the gallium chloride. Wash the ether extracts with two 1-ml. portions of 1:1 hydrochloric acid and discard the washings. Wash out the stem of the separatory funnel with a few drops of 1:1 hydrochloric acid.

Add the ether extract to 0.5 ml. of 10 per cent sodium chloride solution and evaporate to dryness. Use 1-2 ml. of ether to insure complete transfer. Take up the dry residue in 2 ml. of 0.2 *N* hydrochloric acid and use as the sample for determination with 8-hydroxyquinoline by the microtechnic.

Standard

Dissolve 1.000 mg. of pure gallium or 1.230 grams of gallium monoxide in 20 ml. of 1:1 hydrochloric acid and make up to a liter. Each ml. corresponds to 0.001 mg. of gallium. The best concentrations for comparison lie between 0.02 and 0.08 mg. per liter in increments of 0.01 mg., and between 0.08 and 0.2 mg. per liter in increments of 0.02.

Gallium by Quinalizarin

Quinalizarin forms a pink to amethyst lake with gallium in the pH range 4.5-6.0[3] Optimum conditions for identification of as little as 0.02 mg. per liter of gallium by this method are a pH of 5.0; a normal solution of ammonium acetate to increase color intensity; a 2.5 per cent solution of ammonium chloride to heighten the color difference between the blank and the gallium solution; and the presence of 0.5 gram of

[3] H. H. Willard and H. C. Fogg, *J. Am. Chem. Soc.* 59, 40-5 (1937).

sodium fluoride per liter to prevent the formation of a pink color with stannic tin, beryllium, aluminum, thallium, titanium, thorium, rare earths, arsenite, and antimonate ions. The addition of sodium fluoride does not completely inhibit colors produced by quinalizarin with even minute amounts of ferric iron, stannous tin, antimony, copper, lead, indium, germanium, molybdate, vanadyl, and vanadate ions. Nickel and cobalt interfere only by the color of their ions. The iron and lead lakes are blue, whereas those of the other metals are pink. At least 0.5 mg. per liter of zinc must be present before its blue to amethyst color can be seen. The addition of sodium fluoride brings the zinc tolerance to 600 mg. per liter; the zinc-gallium ratio should not exceed 25,000:1. If silver, mercurous mercury, bismuth, tantalum, or columbium are present, they usually either hydrolyze or precipitate as chlorides and can be filtered off. If the concentration of indium does not exceed 8 mg. per liter or of aluminum, 15 mg. per liter, separation is not necessary. If more than 20 mg. of iron or 200 mg. of aluminum per liter is present in the sample solution, the determination of gallium will be inaccurate.

Citrate, oxalate, or tartrate ions prevent the formation of color in all cases. Phosphate when used in excess to repress color due to ferric iron will cause an appreciable decrease in the color due to gallium. A controlled excess of fluoride prevents formation of color due to aluminum but has relatively little effect on colors produced by gallium or iron.

Procedure. Determine the amount of 0.1 *N* sodium hydroxide or hydrochloric acid per 10 ml. of sample to adjust to pH 5.0 with the quinhydrone method. Transfer an amount of sample to contain 0.001-0.005 mg. of gallium to a 50-ml. Nessler tube. To other tubes add suitable gallium standards containing the same salts as are present in the sample from the method of separation used. Adjust sample and standards to pH 5.0 by the predetermined addition. Add to each tube 1 ml. of a 0.01 per cent solution of quinalizarin in ethanol which has been allowed to stand 1-4 days and is preferably not over a week old. Dilute to volume, mix, and after 2 minutes compare.

Gallium by 8-Hydroxyquinoline

If 8-hydroxyquinoline is added to a solution containing gallium at pH 2.6-3.0, a compound is formed. This may be extracted with chloroform for estimation of gallium by the yellowish fluorescence in ultra-

violet light.[4] If indium is present it reacts somewhat at pH 3.0 but not near the lower limits of the range. Aluminum and ferrous ions do not interfere. Cupric, vanadate, and molybdate ions react with the reagent and are extracted but do not fluoresce.

The only other ions found to interfere at less than 1 mg. per ml. were gold 0.1 mg., yttrium 0.06 mg., lanthanum 0.1 mg., titanic 0.05 mg., zirconium 0.05 mg., thorium 0.04 mg., germanium 0.06 mg., columbate 0.02 mg., tellurium 0.02 mg., platinum 0.04 mg. The hydroxyquinolates of cupric, vanadate, molybdate, and ferric ion are extractable by chloroform but do not fluoresce. The precipitate with titanium is not soluble in chloroform. Lithium shows as much fluorescence from 100 mg. as from 0.0001 mg. of gallium but is more greenish. Similarly 10 mg. of beryllium correspond to 0.0001 mg. of gallium. Four mg. of scandium correspond to 0.0001 mg. of gallium. At pH 3.0, 1 mg. of indium corresponds to 0.002 mg. of gallium but is more yellow. Zinc reduces the fluorescence, 20 mg. cutting that of 0.001 mg. of gallium in half. Fluoride reduces the sensitivity of the reaction if an equivalent of aluminum is not also present. Citrate inhibits the reaction, whereas phosphate reduces it slightly.

Procedure. *Macrotechnic.* Take an aliquot which will contain 0.05-1.0 mg. of gallium. Determine with another aliquot the amount of 1:9 ammonium hydroxide or 1:9 hydrochloric acid to adjust to pH 2.6-3.0, and so adjust the aliquot of sample. Alternatively, adjust the pH by addition of 1:1 hydrochloric acid or 1:1 ammonium hydroxide until the full acid color is just shown to methyl orange. Then add 8 per cent sodium acetate solution until the first deviation from the acid color results.

If substantial amounts of iron are present in the ferric form, dissolve 0.5 gram of hydroxylamine hydrochloride per 5 ml. of sample solution. Add 8 per cent sodium acetate solution until a maximum brown color is developed and let stand for 10 minutes. In that time it should become practically colorless. Now adjust the pH to 2.6-3.0.

If vanadate is present, in following the technic for iron, a greenish color will have developed and will disappear on standing. If copper is present, add dropwise a 1 per cent solution of potassium thiocyanate containing 1 per cent of sodium sulfite so long as precipitation of cuprous thiocyanate occurs. Filter and wash the precipitate with a few

[4] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 13, 844-5 (1941); *Anal. Chem.* 19, 63-5 (1947).

ml. of water. If molybdate is present, add a 1 per cent solution of lead nitrate dropwise until precipitation is complete. Filter as for copper.

If less than 0.005 mg. of indium is present per ml., the pH adjustment is sufficient. If more than that amount of indium is present, adjust the pH to 2.6 by addition of a phthalate buffer (Vol. 1, page 173). Transfer the sample and a series of standards to uniform tubes. If zinc is present in the sample add the same amount to the standards.

Prepare a reagent by dissolving 0.1 gram of 8-hydroxyquinoline in 1 ml. of 1:4 acetic acid by warming and dilute to 100 ml. with water. Add 0.25 ml. per 10 ml. of sample and standards. Mix and shake with 0.1 volume of chloroform. Let the chloroform layer separate on the bottom and compare sample and standards with an ultraviolet lamp in a dark room. The fluorescence is yellow with a trace of green, and a blank is essential because chloroform shows a trace of fluorescence. The duplication method can also be applied.

Microtechnic. To the sample in 2 ml. of 0.2 N hydrochloric acid add water to make it about 3 ml. Add 1 ml. of 20 per cent hydroxylamine hydrochloride solution and 6 ml. of a potassium hydrogen phthalate solution containing 4.08 grams per 100 ml. Mix and let stand for about 20 minutes. At this time prepare a blank for later use in duplication of the sample. To sample and standard add 0.25 ml. of 0.1 per cent 8-hydroxyquinoline in a solution containing 0.6 ml. of 1:2 acetic acid per 100 ml. Mix well and add 2 ml. of chloroform. Shake vigorously for a half minute and separate. Match the sample by addition of standard gallium solution to the parallel standard, comparison being by fluorescence in ultraviolet light in a dark room, shaking for a half minute after each addition.

CHAPTER 13

GERMANIUM

Germanium is associated with silver, lead, tin, antimony, and zinc in their sulfide ores. Thus the occurrence is sufficiently rare and of such minor importance that methods of colorimetric estimation are limited. Those used parallel the methods of determination of phosphorus. Fortunately germanium tetrachloride has a boiling point of 86°, which permits its ready distillation from a hydrochloric acid solution and separation. The only other elements so distilled are arsenic and fluorine, the latter as the fluosilicate. Such distillation of arsenic can be avoided by distillation in a current of carbon dioxide.

Samples

Minerals.[1] Weigh a 0.1-gram sample into a platinum dish and add 2 ml. of water, 6 ml. of 1:1 sulfuric acid, 1 ml. of concentrated nitric acid and 10 ml. of 48 per cent hydrofluoric acid. Heat on a hot plate until fumes of sulfur trioxide are given off. Let cool, add a few ml. of water, and again heat to fumes. Repeat this last step twice more. This insures removal of silica, which might otherwise distill as the silicofluoride and later give high results. Transfer to a distilling flask with 35 ml. of 1:1 sulfuric acid and dilute to about 50 ml. Connect with a condenser and fit the flask with an air inlet running nearly to the bottom, a thermometer in the contents of the flask, and a separatory funnel.

To remove fluorides pass a slow stream of air through the solution, heat to 140°, and slowly add water to maintain that temperature while distilling 150 ml Discard this and let the flask cool. Add 15 ml. of water and again heat with air passing. Distill up to 120°, using a paraffined test tube containing 2 ml. of 12 per cent sodium hydroxide solution as receiver. Using the entire contents for the test, neutralize to phenolphthalein with 1:1 hydrochloric acid and treat as a sample for development of molybdenum blue. If there is no color as compared with a blank, removal of hydrofluoric acid is complete. If not, repeat the distillation at 140° and test again, until a negative result is obtained.

[1] Anna-Greta Hybbinette and E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 14, 715-16 (1942).

When hydrofluoric acid removal is complete let the distilling flask cool and add 4 ml. of 1:3 hydrochloric acid. Distill as before with air bubbling through up to 120°, using a test tube containing 4 ml. of 12 per cent sodium hydroxide solution as receiver. Neutralize the distillate with 1:1 hydrochloric acid, transfer to a 25-ml. volumetric flask, and dilute to volume. Repeat the addition of hydrochloric acid and distillation to give a second portion of sample. Unless the sample contains around 0.025 mg. of germanium the first distillate should contain all the germanium. Use both distillates as samples for development of molybdenum blue, and add the results so obtained. If the solutions so obtained are too dilute, concentrate as the sulfide.

Alternatively,[2] treat 10 grams of sample with 20 ml. of water. Add 30 ml. of concentrated hydrochloric acid and distill until 40 ml. of condensate has been collected. All of the germanium has been distilled as the chloride but this technic assumes no fluoride present. Dilute to 50 ml. for use of aliquots as sample for determination by the molybdenum blue method.

Distillation with Arsenic. In distillation of arsenic trichloride (page 183) the germanium goes over at the same time. The technics described are suitable for isolation of germanium except from arsenic.

Concentration as the Sulfide. If the germanium is available in too dilute a solution this furnishes a method of concentration. Approximately neutralize the sample solution. Mix with half its volume of 1:1 sulfuric acid and saturate the solution with hydrogen sulfide. Let stand overnight, filter the germanic sulfide, and wash with 1:100 sulfuric acid saturated with hydrogen sulfide. Discard the filtrate and dissolve the precipitate from the paper with the minimum amount of 1:1 ammonium hydroxide, using a platinum dish as receiver. Add 30 per cent hydrogen peroxide until the germanium is all oxidized to the higher valence, and 1-2 ml. in excess. Boil to destroy excess hydrogen peroxide, let cool, and add 1:5 sulfuric acid until neutral. Dilute to a known volume for taking aliquots.

Standard

If results are to be expressed as germanium use 0.1441 gram of germanium dioxide, if as germanium dioxide use 0.1000 gram. Dissolve the germanium dioxide in a few ml. of water in a 100-ml. volumetric flask

[2] Marcel Orliac, *Compt. rend.* 221, 500-1 (1945).

by addition of a few drops of 12 per cent sodium hydroxide to form sodium germanate. Dilute nearly to volume and neutralize with 1:1 hydrochloric acid. Add a drop or two of excess acid and dilute to volume. Each ml. contains 0.01 mg. of germanium or the dioxide.

Germanium by Molybdenum Blue

Germanate ion condenses with molybdate ion in acid solution to form molybdigermanic acid from which a ferrous sulfate-molybdate reagent produces molybdenum blue.[3] An alternative reducing agent is freshly prepared stannous chloride solution.[4] Phosphorus, silicon, and arsenic yield the same color, hence it is often necessary to isolate germanium by distillation as the chloride. By elimination of fluorides, distillation of fluosilicic acid is avoided. Arsenic distilled with the germanium will only interfere if present in large amounts. The color corresponds to Beer's law up to 4 mg. per liter. All alkaline solutions must be stored in paraffined bottles to avoid contamination with silica.

The developed solution should contain at least 0.004 per cent of ammonium molybdate; increase does not affect the color. The amount of reductant must be carefully controlled. Condensation and color development are relatively stable at around pH 1.0 but color development may also be standardized at somewhat higher pH. The molybdigermanic acid decomposes with time and must therefore be reduced promptly after it is formed.

Barium, bismuth, ferric iron, lead, silicate, or vanadate ion will interfere. Phosphate and fluoride must not exceed 100 ppm. The maximum absorption for photometric reading is at 820-830 mμ.

Procedure. This is designed for development of color in distillates from distillation as germanic chloride. Prepare standards by transfer of 0.5 ml. and 1 ml. of standard containing 0.01 mg. of germanium or the dioxide per ml. to 25-ml. volumetric flasks. To each add 10 ml. of water, 1.5 ml. of 1:1 hydrochloric acid, and 4 ml. of 12 per cent sodium hydroxide solution. Dilute each to volume. Similarly prepare a blank.

Prepare an ammonium molybdate solution containing 6 grams of the tetrahydrate and 16 ml. of concentrated sulfuric acid per 100 ml., and a ferrous ammonium sulfate solution containing 2 grams of the hydrate

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[3] N. S. Poluektov, *Zavodskaya Lab.* **5**, 27-8 (1935); *Mikrochemie* **18**, 48-9 (1935); *Z. anal. Chem.* **105**, 23-6 (1936); Anna Greta Hybbinette and E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* **14**, 715-16 (1942); D. F. Boltz and M. G. Mellon, *Anal. Chem.*, **19**, 873-7 (1947).

[4] Marcel Orliac, *Compt. rend.* **221**, 500-1 (1945).

per 100 ml. acidified with 0.3 ml. of 1:5 sulfuric acid. To prepare the fresh reagent for use add to 50 ml. of water, 10 ml. of the ammonium molybdate, 10 ml. of the ferrous ammonium sulfate, then 25 ml. of 33.7 per cent sodium acetate solution. Dilute to 100 ml. and let stand for 5 minutes.

Transfer 10 ml. of sample, of each standard, and of the blank to 25-ml. volumetric flasks. To each add 2 drops of 12 per cent sodium hydroxide solution to render it alkaline, then acidify with 2 drops of 1:1 acetic acid. To each add 10 ml. of the fresh reagent, and dilute to 25 ml. Let stand for 15 minutes and compare the standards, samples, and blank by reading the transmittance at 830 mμ. The color increases slowly on standing, therefore the time before reading the color must be standardized.

Germanium as Molybdigermanic Acid

Parallel with determination of phosphate not only may germanium be indirectly determined as the molybdenum blue but more directly as the yellow molybdigermanic acid, $GeO_2 \cdot 12MoO_3 \cdot xH_2O$.[5] The color intensity of the complex is constant at above 0.1 gram of ammonium molybdate per 100 ml. The maximum color intensity depends on the acid: with nitric acid it occurs at 0.15-0.3 *N*, with sulfuric acid at 0.15-0.25 *N*, and with hydrochloric acid a sharp maximum occurs at 0.1 *N*. To obtain a color which is stable for 15 minutes it must be developed in approximately 5 *N* acetic acid. If the acidity is over 6 *N* acetic acid, a yellow is developed with the reagent alone. Fluorine weakens the color unless aluminum or zirconium is added to form a complex. Selenium compounds lessen the color unless hydroxylamine hydrochloride is added. Tellurium compounds reduce the color. Citric, oxalic, and tartaric acids reduce the color intensity. Strong reducing agents, such as stannous chloride and sulfide, reduce the compound to the blue cerulomolybdate. The analogous compound is formed with acids of phosphorus, silica, and arsenic. The latter only interferes above 25°. Beer's law is followed up to 75 ppm. of germanium.

Separation from the majority of ions is by distillation. With 5 ppm. of germanium, 2 ppm. of arsenic are tolerated. Addition of more ammonium molybdate or of nitric acid increases this tolerance but reduces the

[5] Charles G. Grosscup, *J. Am. Chem. Soc.* 52, 5154-60 (1930); Robert Schwarz and Hermann Giese, *Ber.* 63B, 2428-32 (1930); I. P. Alimarin and B. N. Ivanov-Emin, *Mikrochemie* 21, 1-10 (1936); R. E. Kitson and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 16, 128-30 (1944).

stability. Because of the instability of the color developed it is desirable to measure the color absorption or to use artificial standards. For the latter, potassium bichromate buffered with 0.5 per cent of borax, or picric acid are suitable.

Procedure. Transfer an aliquot of sample containing 0.5-5 mg. of germanium, which has been distilled as the chloride and concentrated as the sulfide, to a 100-ml. volumetric flask. If not substantially neutral, add 1:1 ammonium hydroxide or 1:1 hydrochloric acid to approximate neutrality. Add 30 ml. of glacial acetic acid, dilute to about 80 ml. and add 10 ml. of a fresh 2.5 per cent solution of ammonium molybdate. Dilute to volume, mix well, and read the color photometrically, compare with a standard prepared at the same time, or compare with a series of artificial standards.

CHAPTER 14

ALUMINUM

As an element constituting about 7 per cent of the earth, and as the most abundant metal, it follows that aluminum is present in many samples. Lacking poisonous properties, the occasions for its determination are not proportionately frequent. Aluminum ion is colorless and forms no colored inorganic compounds of low solubility. Therefore, methods for its determination are limited to salts of high molecular weight organic acids, which are usually dyestuffs. Since the reagent as well as its aluminum lake is colored, the application of photometry can be particularly fruitful. Aluminon possesses some advantages over the other lakes, particularly over alizarin. The anion of the dye is not strongly colored; the molar absorption coefficient is high.[1] Iron is generally separated in preparation of the sample as it interferes to a greater or lesser extent in all methods of determination. This has led to rather general use of the mercury cell for removal of interfering ions.

Samples

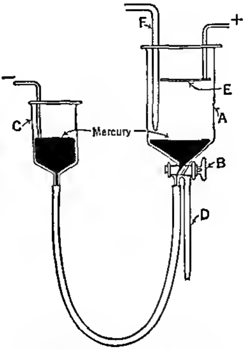
Purification with the Mercury Cell. This equipment is described at this point because it is so commonly used. The original form of Melaven[2] mercury cell is shown in Figure 17. This provides for air agitation of the solution being electrolyzed. An improvement is the motor-driven mercury cell. It is desirable that such a cell have a dual blade stirrer of which one blade stirs the solution being electrolyzed and the other the mercury, thus minimizing the static film at the interface. In operation the cell contains sufficient mercury to provide about 50 ml. in the anode compartment.

A suitable amount of aluminum is 0.01-0.1 mg. in sulfuric acid solution. For samples containing hydrochloric acid or nitric acid evaporate to fumes with sulfuric acid, cool, and dilute. The final solution must be no stronger than 1:4 with sulfuric acid. If not perfectly clear, as when lead sulfate is present, filter the solution before addition to the cell.

[1] A. K. Babko, *J. Applied Chem* (U.S.S.R.) **12**, 1560-7 (1939).
[2] A. D. Melaven, *Ind. Eng. Chem., Anal. Ed.* **2**, 180 (1930).

The intensity of current used may be as great as possible without causing excessive spray or boiling. Usually 3-5 amperes is permissible. This will remove a gram of copper or iron in an hour and a gram of tin, antimony, lead, or zinc in 2-3 hours. In electrolysis, beryllium, vanadium, rare earths, and alkaline earths are not removed and removal of manganese is not complete. The amount of manganese left will usually not interfere with the determination of aluminum.

As an alternative,[3] a suitable cell can be provided by a 400-ml. beaker containing about 30 ml. of mercury. Connect this to the negative terminal of a storage battery or other source of direct current by a mercury-filled, platinum-tipped glass electrode. Provide a platinum gauze or coiled platinum cathode and agitate by air or motor-driven stirrer as for the more complex unit. Remove the final solution by decanting or, preferably, by siphon.



A—Cylindrical glass vessel with conical base.
B—Two-way stopcock.
C—Leveling bulb.
D—Glass tubing constricted in same manner as a burette tip.
E—Platinum gauze or coiled platinum wire.
F—Narrow glass tube for introduction of air to stir the electrolyte (a motor-driven stirrer can be used instead).

FIG. 17

Melaven Type Mercury Cathode Cell

Operation. Transfer the solution to the cell, dilute to about 25 ml. if necessary, and put the electrodes in place. Start the agitating device and turn on the current. Wash down the spray from the sides of the cell 10 minutes before discontinuing the electrolysis.

For discharge from the Melaven type with current still passing, lower the leveling bulb so that the mercury falls just to the upper end of the stopcock bore. Turning the stopcock now shuts off the current and permits discharge of the electrolyte.

It is usual to have to filter out loose floating particles of amalgam. In that case a convenient method is to discharge from D into a filter

[3] "1946 Book of ASTM Methods of Chemical Analysis of Metals," p. 14. American Society for Testing Materials, Philadelphia, Pa.

CHAPTER 14

ALUMINUM

As an element constituting about 7 per cent of the earth, and as the most abundant metal, it follows that aluminum is present in many samples. Lacking poisonous properties, the occasions for its determination are not proportionately frequent. Aluminum ion is colorless and forms no colored inorganic compounds of low solubility. Therefore, methods for its determination are limited to salts of high molecular weight organic acids, which are usually dyestuffs. Since the reagent as well as its aluminum lake is colored, the application of photometry can be particularly fruitful. Aluminon possesses some advantages over the other lakes, particularly over alizarin. The anion of the dye is not strongly colored; the molar absorption coefficient is high.[1] Iron is generally separated in preparation of the sample as it interferes to a greater or lesser extent in all methods of determination. This has led to rather general use of the mercury cell for removal of interfering ions.

Samples

Purification with the Mercury Cell. This equipment is described at this point because it is so commonly used. The original form of Melaven[2] mercury cell is shown in Figure 17. This provides for air agitation of the solution being electrolyzed. An improvement is the motor-driven mercury cell. It is desirable that such a cell have a dual blade stirrer of which one blade stirs the solution being electrolyzed and the other the mercury, thus minimizing the static film at the interface. In operation the cell contains sufficient mercury to provide about 50 ml. in the anode compartment.

A suitable amount of aluminum is 0.01-0.1 mg. in sulfuric acid solution. For samples containing hydrochloric acid or nitric acid evaporate to fumes with sulfuric acid, cool, and dilute. The final solution must be no stronger than 1:4 with sulfuric acid. If not perfectly clear, as when lead sulfate is present, filter the solution before addition to the cell.

[1] A. K. Babko, *J. Applied Chem.* (U.S.S.R.) **12**, 1560-7 (1939).

[2] A. D. Melaven, *Ind. Eng. Chem., Anal. Ed.* **2**, 180 (1930).

Pipet out a 10-ml. aliquot for the mercury cell (page 236). Complete as for acid-soluble aluminum in steel starting at "Use the solution and rinsings. . . ."

Aluminum Steel.[7] The steel may contain 0.04-1.5 per cent of chromium. Decompose a 0.5-gram sample in 5 ml. of 60 per cent perchloric acid and 5 ml. of 1:1 nitric acid. If the steel is low in chromium treat with the nitric acid before adding the perchloric acid. When decomposed, evaporate nearly to dryness to dehydrate silica and precipitate tungsten. Add 25 ml. of water and 10 ml. of concentrated hydrochloric acid, heat to boiling, and filter on a rapid paper. Wash well with hot water or hot 1:100 hydrochloric acid. Discard the residue and evaporate the filtrate and washings to 20 ml. or less.

Separate the iron with isopropyl ether (page 296). To the extracted solution, add 5 ml. of 60 per cent perchloric acid and 2 ml. of concentrated nitric acid. Evaporate to perchloric acid fumes and fume vigorously to insure oxidation of chromium. Cool and add 25 ml. of water. If silica was incompletely removed earlier it will precipitate at this point, in which case remove it by filtration. Neutralize the solution to litmus with concentrated ammonium hydroxide and, if a precipitate forms, just dissolve it with 1:1 hydrochloric acid. Dilute to a known volume and use an aliquot as sample.

Zinc-aluminum-copper and Zinc-aluminum-iron Alloys. A solution was set aside in the determination of copper (page 81). Dilute the solution nearly to 2 liters and add 1:1 sulfuric acid until it is faintly acid. Dilute to volume and as sample for determination of aluminum use 5 ml. of this solution.

Tin.[8] Dissolve a 1-gram sample with 5 ml. of concentrated hydrochloric acid and 1 ml. of concentrated nitric acid in a covered beaker. Wash down the cover glass with 5 ml. of concentrated hydrochloric acid and evaporate substantially to dryness. Add 5 ml. portions of concentrated hydrochloric acid and evaporate to dryness 5 more times. Take up the residue in 10 ml. of 1:30 hydrochloric acid. Heat to boiling and pass in hydrogen sulfide for 5 minutes. Filter and wash the sulfides with 1:100 hydrochloric acid containing hydrogen sulfide. Discard the paper and boil the filtrate until hydrogen sulfide is absent. Add 0.1 ml. of 30

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[7] C. Howard Craft and G. R. Makepeace, *Ind. Eng. Chem., Anal. Ed.* **17**, 206-10 (1945).

[8] S. Yu. Fainberg and T. V. Zaglodina, *Zavodskaya Lab.* **11**, 1109-12 (1945).

which in turn delivers the filtrate and washings into a volumetric flask, later to be diluted to volume for the use of aliquots.

Magnesium Alloys.[4] Dissolve a 0.1-gram sample containing up to 12 per cent of aluminum in 15 ml. of 10 per cent acetic acid. Add water to make 200 ml. of solution of pH approximately 4.0. Use aliquots according to the aluminum content and apply the alizarin method.

Aluminum Oxide in Metallic Aluminum.[5] Dissolve a 1-gram sample of aluminum in 2 ml. of 5 per cent mercuric nitrate solution and 70 ml. of 5 per cent tartaric acid solution. Filter and wash the filter until mercury is completely removed. Discard the filtrate. Ash the paper containing the aluminum oxide in platinum and fuse with a small amount of sodium carbonate-potassium carbonate mixture. Dissolve the melt in about 50 ml. of hot water. Filter and wash the filter. Acidify the combined filtrate and washings with 1:5 sulfuric acid, and use all or an aliquot as sample.

Steel. *Acid-soluble Aluminum.*[6] Dissolve a 1-gram sample containing less than 0.1 per cent of aluminum, or a 0.1-gram sample if above that level, in 25 ml. of 1:9 sulfuric acid and dilute to 500 ml. without filtration. Pipet out a 10-ml. aliquot for the mercury cell (page 236). Use the solution and rinsings as a sample by the Pontachrome Black R method. Omit addition of acetic acid in the procedure as the sulfuric acid already present serves the same purpose.

Acid-insoluble Aluminum. Filter the insoluble residue from the previous sample and wash with 1:20 hydrochloric acid, then with water. Ignite in platinum until carbon is removed and cool. Add 1 ml. of 1:1 sulfuric acid and 5 ml. of 48 per cent hydrofluoric acid. Evaporate to strong fumes of sulfur trioxide. Let cool and wash down the sides of the crucible with a few ml. of water. Evaporate to strong fumes. Let cool and dissolve in 50 ml. of water by warming. Add 10 per cent sodium hydroxide solution to alkalinity to methyl red, then 1:9 sulfuric acid to faint acidity. Add 25 ml. of 1:9 sulfuric acid and dilute to 500 ml.

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[4] F. W. Haywood, F. Harrison and A. A. R. Wood, *J. Soc. Chem. Ind.* 62, 187-9 (1943).

[5] V. P. Okhotin and N. Zubareva, *Zavodskaya Lab.* 2, No. 6, 18-19 (1933).

[6] Alfred Weissler and Charles E. White, *Ind. Eng. Chem., Anal. Ed.* 18, 530-4 (1946); cf. W. Koch, *Tech. Mitt. Krupp, A. Forschungsber.* 2, 37-46 (1938); Paul Roquet, *Rev. mét.* 40, 276 83 (1943).

bearing metal; 1 ml. for spelter, cast bronze, and phosphor-bronze; 2 ml. for brass and journal bearings, and 4 ml. for lead-base bearing metal.

Complete as in the preceding method starting at "Acidify the filtrate with 1:1 hydrochloric acid. . . ."

Solder.[9] Dissolve a 1-gram sample by warming with 30 ml. of concentrated hydrochloric acid. Add 30 per cent hydrogen peroxide dropwise until the color reaches a maximum and evaporate nearly to dryness. Add 5 ml. of 1:1 hydrochloric acid and evaporate just to dryness without overheating. Take up in 10 ml. of 1:3 hydrochloric acid and cool. Filter the lead chloride precipitate and wash with no more than 8 ml of cold 1:3 hydrochloric acid. Add a drop of 30 per cent hydrogen peroxide to the filtrate and evaporate to dryness. Add 5 ml. of concentrated hydrochloric acid and evaporate to dryness. Repeat. Complete as for tin starting at "Take up the residue in 10 ml. of 1:30 hydrochloric acid "

Soft Solders and Babbitt Metal.[10] To a 1-gram sample, add 10 ml. of a solution of 10 ml. of bromine in 90 ml. of concentrated hydrobromic acid and heat on a steam bath. After disintegration is complete, add 5 ml. of 70 per cent perchloric acid and heat with stirring until precipitated bromides have been decomposed. Tin is volatilized as the bromide. If necessary, cool and add 5 ml. of concentrated hydrobromic acid to promote volatilization. Evaporate to 0.5-1.0 ml.

Cool and take up in 15 ml. of water by boiling. Solution may not be complete. Add 3 ml. of 1:9 sulfuric acid which will precipitate the lead. Chill, filter, and wash with cold water until the filtrate is about 25 ml. Discard the precipitate and use the filtrate in a mercury cathode cell (page 236). Evaporate the resulting filtrate and washings to about 25 ml. and cool. Add 1:1 ammonium hydroxide dropwise to alkalinity to litmus, then 1:9 hydrochloric acid to acidity. Use as a sample by the aluminon method.

Silicate Minerals. To a 0.1-0.5 gram sample in a platinum crucible, add 2 ml. of 1:1 sulfuric acid and 10 ml. of 48 per cent hydrofluoric acid Heat to copious fumes and cool. Wash down the sides with about 10 ml. of water and again evaporate to copious fumes. Cool and take up in 100 ml. of water. Neutralize to methyl red with 10 per cent sodium

[9] S. Yu. Fainberg and T. V. Zaglodina, *ibid.* 11, 1109 12 (1945).
[10] Tentative ASTM Method (1946).

per cent hydrogen peroxide and evaporate to 2-3 ml. Add 2-3 ml. of hot water and a drop of methyl red indicator solution. Add concentrated ammonium hydroxide dropwise until the color changes and a drop in excess. Heat below boiling for 20 minutes to coagulate iron and aluminum hydroxides. Filter and wash the residue with 1:100 ammonium hydroxide. Reserve the filtrate for determination of zinc.

Dissolve the precipitated hydroxides from the paper with 1.5 ml. of hot 1:10 hydrochloric acid, added in three portions. Use the flask in which the precipitation was carried out as receiver. Wash the filter well with hot 1:100 hydrochloric acid. Evaporate to 3 ml. and cool.

Add 2 ml. of 10 per cent ammonium thiocyanate solution and 3 ml. of isoamyl alcohol. Shake in a separatory funnel and separate the aqueous layer. Extract again if necessary to remove the balance of the iron. Evaporate the aqueous solution to substantial dryness. Take up the residue in water and use as aample for estimation of aluminum with aluminon.

Bronze. For 0.1 per cent or less dissolve a 1-gram sample, otherwise 0.1 gram, in 10 ml. of 1:1 nitric acid. Add 20 ml. of 1:1 sulfuric acid and evaporate to copious fumes of sulfur trioxide. Let cool, wash down the sides of the beaker with about 15 ml. of water, and again evaporate to atrong fumes. Cool and take up with 100 ml. of water. Neutralize to methyl red with 10 per cent sodium hydroxide solution, then add 25 ml. of 1:9 sulfuric acid. Dilute to 500 ml. and pipet out a 10-ml. aliquot for the mercury cell (page 236). Complete as for acid-soluble aluminum in steel starting at "Use the solution and rinsings. . . ."

Bearing Metals. Dissolve a 1-gram sample in 5 ml. of concentrated nitric acid. Add 30 ml. of 8 per cent sodium hydroxide solution aad separate iron and other metals as the sulfide (page 247). Acidify the filtrate with 1:1 hydrochloric acid and add 2 ml. in excess. Digest at 40-60° until the precipitate settles. Chill if lead is present. Filter and boil the filtrate until all hydrogen sulfide is expelled. Evaporate to 20-30 ml. and filter if necessary. Dilute to a known volume and nse all or an aliquot.

Spelter, Brass, Phosphor-bronze, Lead-base and Tin-hase Bearing Metals. Dissolve a 1-gram sample in 5 ml. of concentrated nitric acid. Add 50 ml. of 8 per cent sodium hydroxide solution for separation of iron as the sulfide (page 247). Add the following amounts of the special sodium sulfide solution described in the method: 0.5 ml. for tin-base

rinse the crucible into the beaker. Add 0.5 per cent sodium hydroxide solution until neutral to a spot test with phenolphthalein. A floc usually indicates approach to the neutral point. Add 1:20 hydrochloric acid to dissolve any floc present, avoiding excess, and transfer to a 100-ml. volumetric flask. Dilute to volume, mix well, and dilute 10 ml. to 100 ml. in another flask. One of these will furnish suitable aliquots for analysis.

This sample is suitable for determination of aluminum, iron, phosphorus, silica, sulfate, calcium, and magnesium.

Leach Liquors.[14] Dilute 10 ml. of clear leach liquor and 15 ml. of concentrated hydrochloric acid to 250 ml. This will bring the aluminum concentration into a suitable range for determination.

Water.[15] To a 50-ml. sample add sufficient 1:1 hydrochloric acid to remove all carbonate hardness and sweep out the carbon dioxide evolved by blowing with air. Use this or an aliquot as sample and if necessary concentrate by evaporation, preferably *in vacuo.*

Biological Material.[16] Suitable samples are 100 ml. of wine, 5-50 grams of tissue, up to 100 ml. of urine, or a 24-hour excretion of feces. Digest with concentrated nitric acid in a Kjeldahl flask, evaporate to dryness in an evaporating dish, and ash at under 500°. Dissolve the carbon-free ash in the minimum volume of concentrated nitric acid and water and transfer to a centrifuge tube. Proceed from "Dissolve 0.1 gram of iron in . . ." later in this technic.

As an alternative method of preparation of the solution of sample [17] dry overnight in a platinum dish at 110°. Dry ashing is somewhat more advantageous than wet ashing because of the lesser amount of salts introduced.[18] Place on a triangle in a cold muffle and heat electrically at such a rate that the temperature is raised to a faint red heat in about 8 hours. Continue at that heat overnight, with a slow stream of oxygen passing into the muffle furnace to complete the ashing. Add 10-25 ml. of concentrated hydrochloric acid, according to the nature of the ash, and 25 ml. of water. Evaporate to dryness to dehydrate silica. Add 10 ml. of 1:5 hydrochloric acid to the ash and boil for 5-10 minutes

[14] Allen L. Olsen, Edwin A. Gee and Verda McLendon, *ibid.* 16, 169-72 (1944).

[15] G Gad, *Kleine Mitt. Mitglied. Ver. Wasser-, Boden-u. Lufthyg.* 15, 126 (1939).

[16] Jacob Cholak, Donald M. Hubbard, and Robert V. Story, *Ind. Eng. Chem., Anal. Ed* 15, 57 60 (1943).

[17] Paul S. Roller, *J. Am Chem Soc.* 55, 2437-8 (1933).

[18] Donald F. Eveleth and Victor C. Myers, *J. Biol. Chem.* 113, 449-65 (1936).

hydroxide solution and add 25 ml. of 1:9 sulfuric acid. Dilute to 500 ml. and pipet out a 10-ml. aliqnot for the mercury cell (page 236). Complete as for acid-soluhle aluminum in steel startiug at "Use the solution and rinsings. . . ."

Alternatively,[11] after volatilizing the silica, evaporate to dryness and fuse with potassium pyrosulfate. Take up in water and use as sample for determination by Eriochrome cyanin-R. If iron and manganese are high they may require removal. Titanium may he removed hy hydrolysis.

Carbonate Minerals. Ignite a 1-gram sample at ahout 1100° for 30 minutes. Cool and dissolve in 40 ml. of 1:1 hydrochloric acid. Filter and wash the residue. Ignite the residue in platinum and cool. Add 5 drops of 1:4 sulfuric acid and 5 ml. of 48 per cent hydrofluoric acid. Evaporate to dryness and fuse with 0.5 gram of potassium pyrosulfate. Take up in water and combine with the original filtrate. Add 10 grams of ammonium chloride, and 1:1 ammonium hydroxide to alkalinity to methyl red. Heat to coagulate, filter, and wash. Dissolve the residue in 25 ml. of 1:9 sulfuric acid and dilute to 500 ml.

Pipet out a 10-ml. aliquot for the mercury cell (page 236). Complete as for acid-soluble aluminum in steel starting at "Use the solution and rinsings. . . . "

Quartzite.[12] Heat a 0.3-gram sample in a platinum crucible with 5 ml. of 48 per cent hydrofluoric acid and about 0.2 gram of oxalic acid. When dry, add 0.5 ml. of 5 per cent oxalic acid solution and again evaporate to dryness. Aluminum is present as the oxide. Fuse the residue with a small amount of potassium pyrosulfate, take up in water and use all or an aliquot as sample.

Boiler Scale.[13] Grind a representative sample to a very fine powder and dry at 105°. Weigh out 0.040 gram and heat slowly to 950-1000°, then for 45 minutes or until the weight is constant. Cool in a desiccator, cover the sample with 0.2 gram of potassium carbonate, and fuse. When cool, set in a heaker, add 5-ml. of 1:40 hydrochloric acid, and heat on a hot plate for 4-6 minutes. Dnring that time a floc usually forms and the melt is separated from the crucible. Add 10 ml. more of the acid and heat until solntion is complete. Add further acid if essential. Cool and

[11] F. Richter, *Z. anal. Chem.* 127, 113-39 (1944).

[12] N. O. Zeldin, *Ogneupory* 7, 560-4 (1939).

[13] F. K. Lindsay and R. G. Bielenberg, *Ind. Eng. Chem., Anal. Ed.* 12, 460-3 (1940).

rinse the crucible into the beaker. Add 0.5 per cent sodium hydroxide solution until neutral to a spot test with phenolphthalein. A floc usually indicates approach to the neutral point. Add 1:20 hydrochloric acid to dissolve any floc present, avoiding excess, and transfer to a 100-ml. volumetric flask. Dilute to volume, mix well, and dilute 10 ml. to 100 ml. in another flask. One of these will furnish suitable aliquots for analysis.

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[14] Allen L. Olsen, Edwin A. Gee and Verda McLendon, *ibid.* **16**, 169-72 (1944).

[15] G Gad, *Kleine Mitt. Mitglied. Ver. Wasser-, Boden u. Lufthyg.* **15**, 126 (1939).

[16] Jacob Cholak, Donald M. Hubbard, and Robert V. Story, *Ind. Eng Chem., Anal. Ed.* **15**, 57 60 (1943).

[17] Paul S. Roller, *J. Am. Chem. Soc.* **55**, 2437-8 (1933).

[18] Donald F Eveleth and Victor C. Myers, *J. Biol. Chem.* **113**, 449-65 (1936).

Transfer to a tube and centrifuge for 5 minutes. Decant the solution into a centrifuge tube and if the residue is carbon-free, discard it. If not, transfer to a platinum crucible and dry. Volatilize the silica with hydrofluoric and sulfuric acids and ignite until free of acid and carbon. Fuse in a 1:1 mixture of sodium and potassium carbonates and dissolve in the sample solution.

Dissolve 0.1 gram of iron in a minimal amount of 1:4 hydrochloric acid, agitate to permit air oxidation to ferric chloride, and dilute to 100 ml. About 1 mg. of iron is needed for entrainment of 0.05-0.075 mg. of aluminum as phosphate. Since the iron will later have to be removed, excessive amounts are undesirable. Blood samples contain sufficient iron so that addition is not necessary. Add 1 ml. of this iron solution, more if necessary because of the high aluminum content, 1 ml. of saturated solution of ammonium acetate, and 5 ml. of 4 per cent diammonium phosphate solution. For urine samples the normal phosphate content is sufficient. Large amounts of added phosphate lead to high blanks but excess must be present. Dilute to 20 ml., mix well, and add 6 drops of a 0.1 per cent solution of bromocresol green. If necessary to insure complete solubility of phosphate at this stage add a few drops of 1:1 hydrochloric acid. Add 1:2 ammonium hydroxide dropwise until the indicator shows pH 4.2 by comparison with a standard buffer (Vol. 1, p. 174).

Dilute to 30 ml. and heat the tube in boiling water for about 30 minutes. Wash down the sides of the tube with a fine jet of hot water and centrifuge for 10 minutes. The aluminum will have been carried down as the phosphate with the ferric phosphate serving as collector. Discard the supernatant layer. Wash down the sides of the tube with hot water, break up the precipitate, and dilute to about 20 ml. Heat in a boiling water bath for 10 minutes to coagulate the precipitate, then centrifuge for 10 minutes and discard the supernatant liquid. If excess phosphate is incompletely removed in washing, it can cause later precipitation of aluminum phosphate in the estimation with consequently low values.

Dissolve the precipitate in 5 ml. of 1:10 sulfuric acid by heating in a boiling water bath. Dilute to about 10 ml. and throw down the silica by centrifuging. Remove the iron by extraction as the cupferron complex (page 247). Use the final solution so obtained. Not over 0.05-0.075 mg. of aluminum may be present in 100 ml. for determination or it will result in precipitation of the phosphate. Aluminon or alizarin S is the preferred reagent for determination.

Foods.[19] Ash a sample of 3-5 grams in a quartz crucible below 450°. Dissolve the ash in a small volume of 1:1 hydrochloric acid and evaporate to dryness on a water bath. Take up the residue in water, transfer to a tube, and centrifuge to remove silicic acid. Decant the liquid into another tube and add 1 ml. of 30 per cent acetic acid and about 0.25 gram of ferric chloride. Then add 2 ml. of a 1 per cent solution of 8-hydroxyquinoline in acetic acid. Shake vigorously and add 2 ml. of saturated sodium acetate solution. Heat in a water bath at 50° for 10-15 minutes. The iron serves as collector since less than about 0.05 mg. of aluminum would not separate satisfactorily. Centrifuge and discard the liquid. Disperse the precipitate in water, centrifuge again, and discard the wash water. Dissolve the precipitate in a few drops of concentrated nitric acid, transfer to a quartz crucible, and evaporate to dryness on a water bath. Ash below 500° and let cool. Add 1 ml. of 1:10 hydrochloric acid and heat on a water bath to evaporate excess acid. Take up in 2-3 ml. of 1:12 hydrochloric acid and transfer to a separatory funnel. Extract the iron as the thiocyanate (page 247) and use all or an aliquot as sample.

Plant Tissue.[20] Weigh a suitable sample and treat with a mixture of equal parts of concentrated hydrochloric acid and 60 per cent perchloric acid, adding enough to make a semiliquid mixture. Warm gently until organic matter has been destroyed. Evaporate almost to dryness and take up in 5 ml. of 1:20 hydrochloric acid. Dilute with 15 ml. of water and add a drop of methyl red indicator. Neutralize with 1:1 ammonium hydroxide and add 1 ml. of saturated ammonium acetate solution. Transfer to a centrifuge tube, heat in boiling water for 10 minutes to precipitate the basic acetates, and centrifuge. Decant the upper layer and dissolve the precipitate in 1 ml. of 1:1 hydrochloric acid. Use for separation of iron as the sulfide (page 247).

Save the iron precipitate for a separate determination of that element. Neutralize the aluminum solution with 1:1 hydrochloric acid and boil until all hydrogen sulfide has been removed. Use as sample, aliquoting if desirable.

Separation from Calcium, Magnesium, and Iron. Measure out a portion of the sample which will contain 0.02-0.5 mg. of aluminum. This solution may also contain calcium, magnesium, and iron. Transfer to a

[19] L. M. Kul'berg, *Voprosy Pitaniya* 8, No. 5, 75-9 (1939); L. M. Kul'berg and E. I. Rovinskaya, *Zavodskaya Lab.* 9, 145-9 (1940).

[20] H. Steudel, *Biochem. Z.* 253, 387-94 (1932).

centrifuge tube and add a few crystals of ammonium sulfate. When these are dissolved, add 1 ml. of a 30 per cent solution of urotropine. Heat to boiling for 1 minute over a small flame and centrifuge for 3 minutes. Remove the supernatant liquid and add 2 ml. of water. Stir well and heat to boiling. Centrifuge and remove the wash water. The precipitate so obtained contains the aluminum and iron. Discard the decantates.

Add 1 ml. of 2 per cent sodium hydroxide solution to the precipitate. If more than 0.05 mg. of aluminum is present, also add 1 ml. of water. Heat to boiling and centrifuge. Separate the upper layer, which contains the lauminum, and transfer to a round-bottom centrifuge tube. Wash the iron residue twice with 1-ml. portions of hot water, heating to boiling with each. The total volume of aluminate solution and washings to be used as sample should be 3-4 ml. For the majority of methods add 1:1 sulfuric acid dropwise to faint acidity.

Separation of Iron. Various technics for separation of iron from aluminum are used. Because of their rather general applicability they are concentrated here rather than being given, and often necessarily repeated, with specific samples.

With Isopropyl Ether. This was developed for the large amounts of iron in steel samples.[21] If necessary concentrate to under 20 ml. Transfer the solution to a separatory funnel and dilute to 20 ml. with 1:1 hydrochloric acid. Use 10 ml. of concentrated hydrochloric acid and 30 ml. of isopropyl ether to rinse the original container and add this to the separatory funnel. The permissible range of acidity is 6.5-8.5 *N.* Shake cautiously to extract iron, cooling if necessary to keep the temperature below 30°, and release the pressure. Shake vigorously for 10 seconds and let the layers separate. Draw off the aqueous layer into a second separatory funnel. Wash the ether layer with 2 ml. of 2:1 hydrochloric acid and add these washings to the aqueous layer. Repeat the extraction using 10 ml. of isopropyl ether and washing it with 1 ml. of 2:1 hydrochloric acid. Extract again. This third ether layer should be colorless and free of iron. If it is not, continue to extract until no more iron remains. Each extraction can be expected to be over 99 per cent efficient. Discard the extracts. Boil the aqueous layer to eliminate dissolved isopropyl ether.

[21] C. Howard Craft and G. R. Makepeace, *Ind. Eng. Chem., Anal. Ed.* 17, 206-10 (1945).

As the Sulfide. This will ordinarily be applied to an acid solution of an alloy. Neutralize with 8 per cent sodium hydroxide and add 2 ml. in excess.

Heat to boiling, rotating over a free flame. Prepare a sodium sulfide solution by saturating 20 ml. of 8 per cent sodium hydroxide solution with hydrogen sulfide and adding 20 ml. of the same sodium hydroxide solution. Add 20 ml. of this sodium sulfide solution to the solution of the sample, swirl for a few minntes, and filter the resulting solution of sodium aluminate.

Precipitation of hydroxides as a method of separation from aluminum is not very satisfactory due to sorption of aluminum and precipitation of double compounds, but the transition from hydroxide to sulfide in the solid phase removes this source of loss.

As the Cupferron Complex.[22] Adjust the acidity of the sample to about 1:5 with sulfuric acid and the volume to about 25 ml. Up to 10 mg. of iron may be present but nitric acid must be absent. Add 2.5 ml. of 6 per cent solution of cupferron and shake with 10 ml. of chloroform to extract the iron-cupferron complex. Repeat extractions with 10 ml., 5 ml., and 5 ml. of chloroform. Discard the extracts. Boil the aqueous solution until chloroform is eliminated and add a drop of methyl red indicator solution. Add 1:1 ammonium hydroxide to alkalinity, then a drop of 1:1 hydrochloric acid to restor acidity, and 5 ml. of 1:1 hydrochloric acid. Bleach the indicator by addition of 2 drops of saturated bromine water. Add 0.5 ml. of 10 per cent hydroxylamine hydrochloride solution and dilute to an appropriate volume.

As the Thiocyanate. Adjust the acidity to approximately that of 1:12 hydrochloric acid. To extract the iron, add 0.5 ml. of a 50 per cent solution of potassium thiocyanate and 10 ml. of freshly distilled ether. Shake, let separate, and withdraw the lower layer to another funnel. Repeat the extraction with 0.2 ml. of thiocyanate solution and 10 ml. of ether Continue until both the aqueous and ether layers are colorless.

Transfer the aqueous solution of aluminum to a tube and carefully evaporate the dissolved ether.

Standard

Dissolve 0 9286 gram of alum, $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$, in water and dilute to 1 liter Dilute 10 ml. of this solution to 1 liter. The resulting solution contains 0.001 mg. of aluminum oxide per ml. If results are to be expressed as aluminum, similarly use 1.757 grams of alum.

[22] N. Strafford and P. F. Wyatt *Analyst*, **72**, 54-6 (1947).

liter. The color developed may intensify for a few days, then become stable if protected from light.

Transfer an aliquot of sample containing 0.02-0.1 mg. of aluminum to a 50-ml. Nessler tube and a similar standard to another tube. Adjust the salt content of the standard to match the sample. Dilute each to about 30 ml. To each add 5 ml. of concentrated hydrochloric acid, 5 ml. of glacial acetic acid, and 2.5 ml. of the prepared reagent solution. Mix, and slowly add concentrated ammonium hydroxide saturated with ammonium carbonate.

Add 5 ml. of glacial acetic acid, mix, and let stand for 10 minutes. Again neutralize with concentrated ammonium hydroxide saturated with ammonium carbonate. This time add 5 ml. in excess. Let the solution stand for a few minutes and compare by balancing. Alternatively read the transmittance at 520 mμ and compare with a standard curve.

Alkaline Solution. Transfer a sample containing 0.01-0.03 mg. of aluminum to a 25-ml. flask. Approximately neutralize excess acidity or alkalinity. The volume must not exceed 15 ml. at this point. Add 1 ml. of 1:9 hydrochloric acid and 1 ml. of a 0.2 per cent aqueous solution of aluminon. Dilute to about 20 ml. and add 2 ml. of 25 per cent ammonium acetate solution to bring the pH to about 5.5. Add 1 drop of 1:1 ammonium hydroxide every 2 seconds until 2 ml. are added. The cloudy precipitate of the dye-acid will first dissolve so that the solution is clear. Cut the rate of addition in half and continue until a floating piece of litmus paper turns blue. The color developed is a function of the rate of addition of ammonium hydroxide. Reacidifying later will eliminate minor deviations in this factor. Dilute to volume and mix. Read the transmittance after 15 minutes with a filter centering around 520 mμ and compare with a calibration curve.

Aluminum by Eriochrome Cyanine-R

Eriochrome cyanine-R, one of the triphenylmethane dyes, is orange red, and forms a stable, water-soluble, violet-red lake with aluminum.[26] Three mols of reagent react with one of aluminum. At pH 5.4-6.0 it requires about 3 days for full color development. Therefore when

[26] I. M. Kolthoff, *J. Amer. Pharm. Assoc.* **17**, 360 1 (1928); F. Alten, H. Weiland and E. Knippenberg, *Z. anal. Chem.* **96**, 91-8 (1934); F. Alten, B. Wandrowski and E. Hille, *Angew. Chem.* **48**, 273-5 (1935); Theodor Millner, *Z. anal. Chem.* **113**, 83-118 (1938); Walter Koch, *Arch. Eisenhüttenw.* **12**, 69-80 (1939); K. Steinhäuser, *Aluminum* **24**, 176-8 (1942); August Rauch, *Z. anal. Chem.* **124**, 17-25 (1942).

operating at that pH, the period after addition of the reagent must be rigorously standardized.[27] At pH 3.8 the constant depth of color is reached in 4 hours at room temperature or 1 hour at boiling. Standard curves must be prepared with the same lot of reagent used for development of color as there are variations. Old solutions and the presence of salts alter the intensity.

Lakes are also formed with nickel, zinc, manganese, chromium, iron, and magnesium. The effect of iron or manganese is seven times that of aluminum, that of magnesium double that of aluminum. Phosphate and organic materials decrease the color. The solution is usually buffered to pH 4.6-5.6 for maximum color development. Thus the interferences are very much like those for aluminon and similar technics for purification of the test solution are applicable. The method is accurate to better than 6 per cent in the absenee of iron or manganese. Beer's law holds only over a relatively short range, so comparison must be with a very similar standard. Also the reagent introduces a yellow color which must be allowed for. Photometrically the maximum is at 530 mμ which therefore requires the use of ultraviolet from a mercury lamp. Practically, a 578 mμ filter is used.

Procedure. Transfer a sample, which has been freed from interfering ions, to a 50-ml. volumetric flask. This should contain 0.001-0.01 mg. of aluminum. Dilute to about 25 ml. Add 2 ml. of a 0.1 per cent solution of Eriochrome cyanine-R, and 1 ml. of 1 per cent acetic acid. Mix and let stand for 15 minutes. Then titrate with 8 per cent sodium hydroxide solution until the color is precipitated. Titrate with 1:100 acetic acid until the maximum yellow or red color is developed. Add 15 ml. of a buffer solution for pH 5.2-5.4 containing 14.2 grams of sodium acetate and 4.0 grams of acetic acid per liter. Let stand for 15 minutes and dilute to volume. Read the transmittance with a 578 mμ filter and compare with a calibration curve.

Aluminum by Hematoxylin

Application of the hematoxylin method is largely confined to water and soil extracts.[28] At a pH of 8.2 the dye is sorbed on an aluminum

[27] F Richter, *Z. anal. Chem.* **126**, 426-52 (1944).

[28] W. D. Hatfield, *Ind. Eng. Chem.* **16**, 233 (1924); Georg Gad and Kate Naumann, *Gas u. Wasserfach* **80**, 58 9 (1937); *ibid.* **81**, 164 (1938); Harold W. Knudson, Villiers W. Meloche and Chancy Juday, *Ind. Eng. Chem., Anal Ed.* **12**, 715-18 (1940); G. U. Houghton, *Analyst* **68**, 208-11 (1943); V. Ya Tartakovskii, *Zavodskaya Lab.* **9**, 971-5 (1940).

Aluminum Fluorimetrically by Pontachrome Blue Black R

A very sensitive reaction of aluminum ion is the formation of a red fluorescence with Pontachrome Blue Black R, 4-sulfo-2-hydroxy-α-naphthalene-azo-β-naphthol, color index 202.[30] It follows that a fluorometer is required. One mol of dye combines with one of aluminum. Corning filter 5874 is suitable for isolation of the energizing radiation. A filter centering around 360 mμ is suitable for isolation of the fluorescence. The optimum fluorescence is around pH 4.8 with 1.5 ml. of 0.1 per cent solution of the reagent per final volume of 50 ml. More reagent causes rapid decrease in fluorescence in some ranges. Full color development occurs in less than an hour at room temperature. Decrease of temperature has an unimportant effect on the total color fluoresced. The fluorescence increases almost linearly with aluminum concentration up to 1 ppm. of aluminum and remains practically constant thereafter.

The reagent is also known commercially as Superchrome Blue Black. Pontachrome Violet SW, color index 169, may be used as an alternative.[31] Even a trace of ferric ion destroys the fluorescence. Interfering ions must be removed by electrolysis with a mercury cathode (page 236) in order to apply this method. Even then titanium may interfere.

Procedure. Transfer a sample containing not over 0.05 mg. of aluminum and preferably about 0.02 mg. to a 50-ml. Nessler tube. Dilute to about 40 ml. and add 5 ml. of 10 per cent ammonium acetate solution and 1 drop of glacial acetic acid. Mix and add 1.5 ml. of 0.1 per cent solution of Pontachrome Blue Black R in ethanol, which has stood several days before using. Dilute to volume and let stand for 1 hour. Read the fluorescence through a red filter and compare with a curve produced from standards.

Aluminum by Alizarin-S

The difference in color of alizarin monosulfonic acid and the aluminum lake of the dye permits colorimetric estimation in acid solution.[32]

[30] C. E. White and C. S. Lowe, *Ind. Eng. Chem., Anal. Ed.* 9, 430-1 (1937); Alfred Weissler and Charles E. White, *ibid.* 18, 530-4 (1946).

[31] J. A. Radley, *Analyst* 68, 369-70 (1943).

[32] F. W. Atack, *J. Soc. Chem. Ind.* 34, 936 (1915); A. P. Musakin, *Zavodskaya Lab.* 3, 1085-9 (1934); *J. Applied Chem. (U.S.S.R.)* 9, 1340-6 (1936); *Z. anal. Chem.* 105, 351-61 (1936); S. N. Rozanov and G. A. Markova, *Zavodskaya Lab.* 4, 1023-31 (1935); A. K. Babko, *Univ. état Kiev, Bull. sci., Recueil chim.* 2, No. 2, 59-68 (1936); Valter Öhman, *Tek. Tid.* 71, No. 28, Uppl. A-C, *Kemi* 56-7 (1941).

This is the original method of laking out aluminum and reading the color of the dispersed colloid.

The reaction tolerates fair amounts of calcium, magnesium, or zinc salts although they can cause high values. Iron must be absent because it cannot be corrected by citrate complex formation. No more than a trace of chromium, cobalt or manganese is permissible. Each 100 ml. of sample solution may contain no more than 2 mg. of copper, 2 mg. of sulfide, 2 mg. of stannic ion, 20 mg. of nickel ion or 20 mg. of nitrate ion. The most favorable pH is 3.6, readily obtained by acetic acid buffered with sodium acetate or ammonium acetate. The color takes time to develop and therefore the duplication method is not applicable. When obtained it is stable for 10-15 minutes, and under some conditions for many days.

Such a compound is a sorption complex and it follows that the composition will vary with time, temperature, pH, and concentration of reagent. The extent of conformity to Beer's law varies with different types of samples. For maximum accuracy comparison should be photoelectric or with a standard differing by no more than 10 per cent from the sample.

A colloidal stabilizer such as gum arabic or starch glycerite is often added. Accuracy to ±2 per cent is a reasonable expectation.

Procedure. Transfer an aliquot of sample containing 0.01-0.10 mg. of aluminum to a 100-ml Nessler tube. In a similar tube take an equivalent standard containing the same salts and acid content. Adjust the acidity of each to approximately 1 ml. of concentrated hydrochloric acid in each. Dilute to about 60 ml. and add 10 ml. of 0.1 per cent solution of alizarin S as a maximum. This may be judiciously reduced for samples near the lower range of the concentration. At this point the solution should be yellow. Add 4 ml. of 1:2 ammonium hydroxide with mixing. This should convert the color to orange. Let the tubes stand for 10 minutes and add 10 ml. of 1:2 acetic acid. Mix, dilute to volume, mix well, and let stand for 20 minutes before comparing.

For small samples use one-half, one-quarter, or one-tenth the above amounts. For photoelectric examination of the sample, color filters used are 470, 500, 530, 580, and 603 mμ. The greatest difference between reagent and the aluminum lake is around 580 mμ.

Aluminum by Morin

Another color lake which can be used for fluorometric estimation of aluminum is that formed with the dyestuff principle of fustic known as

and bismuth give precipitates at the hydrogen-ion concentration used, but may be prevented from interfering by the addition of sodium tartrate or Rochelle salt.

As buffer mix 10 parts of 5 *N* acetic acid with 9 parts of 5 *N* ammonium hydroxide. On diluting tenfold with the sample solution this gives a pH between 5.4 and 5.8. For the determination, to 10 ml. of a neutral solution of aluminum, and to a series of comparable standards, add 0.25 to 1 ml. of buffer solution, then 0.3 ml. of a 0.1 per cent solution of quinalizarin in 95 per cent ethanol. Shake and compare after 15 or 30 minutes.

The color of aluminum with 8-hydroxyquinoline may be directly determined by chloroform extraction from solution. The maximum absorption is at 395 mμ.[39] The reagent gives a slight but measurable absorption. Complete extraction is possible only in the pH range 4.3-4.6, and then only with difficulty. Deviations from Beer's law are insignificant. Complete extraction of the corresponding iron complex occurs at a lower pH, so that it can be extracted prior to the aluminum complex. Another indirect method is to use the solution of the well-washed precipitate to reduce phosphomolybdotungstic acid, Folin's reagent.[40] Zinc and bismuth must be absent.

For the determination acidify 1-4 ml. of the solution of sample to about pH 3.0. Add 0.5 ml. of saturated sodium acetate solution and 4 drops of a 0.5 per cent solution of hydroxyquinoline acetate. Heat on a water bath 15 minutes to cause the precipitate to settle. When cool dilute to about 5 ml. and centrifuge for 10 minutes at 2000-2500 rpm. Decant the clear liquid. Stir up the precipitate with 5 ml. of water and centrifuge again. Repeat with 2 ml. of water.

Dissolve the precipitate in 1 ml. of 1:2 hydrochloric acid and rinse into a 25-ml volumetric flask with 15 ml. of water. Add 1 ml. of Folin's reagent (page 623) and 6 ml. of cold saturated sodium carbonate solution. Dilute to volume and compare after 30 minutes with a standard similarly prepared at the same time.

By adding cupferron, the ammonium salt of nitrosophenylhydroxylamine, to a neutral or faintly acid solution of aluminum salt, a colloidal solution yellow to transmitted light and bluish to reflected light is obtained with a concentration below 3 mg. of aluminum per

[39] Therald Moeller, *Ind. Eng. Chem., Anal. Ed.* 15, 346-9 (1943); C. H. R. Gentry and L. G. Sherrington, *Analyst* 71, 432-8 (1946).

[40] M. Teitelbaum, *Z. anal. Chem.* 82, 366-74 (1930).

liter.[41] The method is applicable in the presence of any substance not precipitated by cupferron in faintly acid solution such as magnesium, silver, divalent mercury, pentavalent antimony, tri- and pentavalent arsenic, lead, cadmium, zinc, manganese, nickel, cobalt and chromium. Trivalent chromium ion begins to produce turbidity at a concentration of about 5 mg. per liter. The last four of the series give colored solutions and in their presence aluminum can only be determined nephelometrically. Copper, tin, trivalent antimony, bismuth, and iron interfere as they also give very slightly soluble derivatives with cupferron. The procedure differs slightly according to the concentration of aluminum. The method has been used with an average error of less than 1 per cent and a maximum error of 3.2 per cent. Beer's law holds. The method is probably applicable to other ions forming difficultly soluble compounds with cupferron such as copper, tin, trivalent antimony, bismuth, and iron.

Add to 25 ml. of sample solution and an equivalent standard, 1 ml. of freshly prepared 5 per cent aqueous solution of cupferron and 1 ml. of a 0.1 per cent solution of gelatin. Compare in the colorimeter, preferably by superimposing a piece of blue glass between the eye and the ocular to increase the sensitivity.

Fluorescent methods have been given with Pontachrome Blue Black R and Pontachrome Violet SW. Other dyestuffs which give lakes with aluminum suitable for colorimetric estimation are Solochrome Violet RS, Soledon Golden Yellow RKS, and Solocet Violet RS.[42] An alternative fluorescent method is by use of quercetin.[43]

[41] L. de Brouckère and E. Belcke, *Bull. soc. chim. Belg.* 36, 288 (1927); Caupolican Jorge Pereyra, *Bol. obras sanit. nacion* (Buenos Aires) 3, No. 22, 389 97 (1939)

[42] J. Haslam and G. P. Alcock, *Analyst* 70, 335-6 (1945).

[43] A. L. Davydov and V. S. Devekki, *Zavodskaya Lab.* 10, 134-8 (1941).

volumetric flask, make up to volume, and mix. Filter and use aliquots, preferably by the p-nitrobenzeneazoöreinol method.

Alternatively, fuse 0.2 gram of sample with 2 grams of sodium carbonate, cool and take up with 10 ml. of 1:1 hydrochloric acid. Evaporate to dryness to insolubilize silica. Take up with 20 ml. of hot 1:20 hydrochloric acid and filter. Wash the filter with hot 1:20 hydrochloric acid. Add 8 per cent sodium hydroxide solution dropwise until the filtrate is alkaline. If iron is present it will be precipitated as ferric hydroxide. Dilute to 100 ml., let the precipitate settle, and take an aliquot of the supernatant liquid.

Separation from Aluminum.[6] To separate aluminum from a solution containing about 0.1 gram of aluminum and beryllium, acidify slightly with 1:1 sulfuric acid. Dilute with hot water to 500 ml. Warm a cold, saturated solution of ammonium acetate containing 3 grams of pure gallotannic acid per 100 ml. to about 80°. The solution must remain clear. Add a slight excess of this, with stirring, to the aluminum-beryllium solution. An aluminum hydroxide-tannin compound separates at once. Boil for 2 minutes, then let cool. Test the completeness of the precipitation in the supernatant liquid with a few drops of reagent. Filter and wash with a warm 5 per cent solution of ammonium acetate.

Heat the filtrate and washings containing the beryllium to boiling. Add concentrated nitric acid dropwise to oxidize the tannin until the solution becomes colorless. Dilute to a known volume and use aliquots.

Standards

Dissolve 0.2774 gram of beryllium oxide in 10 ml. of concentrated sulfuric acid. Transfer to a liter volumetric flask containing about 200 ml. of water. Dilute to volume. Alternatively dissolve 1.9638 gram of beryllium sulfate tetrahydrate, $BeSO_4 \cdot 4H_2O$, in water and dilute to 1 liter. Either solution contains 0.1 mg. of beryllium per ml.

Beryllium by p-Nitrobenzeneazoörcinol

If a bright yellow, slightly alkaline solution of p-nitrobenzeneazoörcinol is added to a solution containing beryllium, a red-brown lake forms instantaneously.[7] This color change is used as the basis for a colorimetric method of determination The zone of optimum alkalinity

[6] Ludwig Moser and Moritz Niessner, *Monatsh.* **48**, 113-21 (1927).
[7] A. S. Komarovskii and N. S. Poluektov, *Mikrochemie* **14**, 315-17 (1934).

is narrow, and boric acid may be used as a buffer to improve reproducibility. The transmittance of blanks decreases with increasing alkalinity and temperature, but the transmittance of beryllium solutions increases with temperature.

Aluminum, up to 9.6 grams per liter, exerts little influence, provided an excess of 1 mol of sodium hydroxide is added per mol of aluminum present. Zinc may be removed as the sulfide, although about 80 times as much zinc as beryllium may be present without interference. If copper is present, even in minute quantities, it must be removed. Use 488-525 mμ filters for determination of the transmittance.[8] Good results are obtainable with 0.005-18.0 per cent of beryllium in a 0.1-0.6 gram sample.

Procedure. To prepare the dye, dissolve 1.38 grams of *p*-nitroaniline in 5 ml. of 1:1 hydrochloric acid. In an ice bath at 0°, add an equally cold concentrated solution of 0.85 gram of potassium nitrite in water. Maintain the temperature of the resulting solution of *p*-nitrobenzene diazonium chloride at 0°, and add a cold solution of 1.42 grams of orcinol, made alkaline with sodium carbonate solution. Acidify the mixture and filter the bright red crystals which separate. Wash with 1:20 hydrochloric acid, follow with water, and dry. To prepare the reagent solution, dissolve 0.025 gram of *p*-nitrophenylazoörcinol in 100 ml. of 0.4 per cent sodium hydroxide solution. Stir mechanically for several hours.

Transfer an aliquot of sample representing 0.0002-0.001 mg. of beryllium to a 25-ml. measuring cylinder. Taking into account only the excess sodium hydroxide and the alkalinity of the dye solution, adjust the alkali content to 3 ml. of 8 per cent sodium hydroxide. Add 5 ml. of 4 per cent boric acid solution, dilute to about 18 ml., and add 6 ml. of reagent. Dilute to 25 ml., mix, and measure the transmittance.

Beryllium by Morin

Morin, a tetrahydroxyflavanol, in sodium or potassium hydroxide solution gives a sensitive yellow-green fluorescence,[9] measurable preferably under ultraviolet light, which may be made specific for beryllium.[10] The reaction is more sensitive than that with quinalizarin.[11] Morin

[8] W. Stross and G. H. Osborn, *J. Soc. Chem. Ind.* 63, 249-51 (1944).

[9] H. L. J. Zermatten, *Proc. Acad. Sci. Amsterdam* 36, 899-900 (1933); Giovanni Venturello, *Atti reale accad. sci. Torino, Classe sci. fis., mat. nat.* 79, 263-8 (1943-4).

[10] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 12, 674-5, 762-4 (1940).

[11] H. Fischer, *Z. anal. Chem.* 73, 54-64 (1928).

itself sbows a faint yellow brown fluorescence in ultraviolet light, limiting the sensitivity of the method. An increase in the sodium bydroxide concentration decreases the sensitivity of the reactiou. In 4 per cent sodium hydroxide solution, 0.1 ppm. of heryllium in hright daylight, and 0.02 ppm. under ultraviolet light, may he determined.

Large amounts of eopper, silver, gold, manganese as manganate, aud chromium interfere by oxidizing the morin in alkaline solution. Lithium, calcium, zinc, and scandium give a fluorescence similar to that of heryllium. The fluorescence of lithium and calcium is not discernible in daylight hut is visihle under ultraviolet light. The addition of sodium pyrophosphate will prevent fluorescence of calcium in ultraviolet light hut if a large amount of calcium is present the pyrophosphate solution becomes turhid. As little as 1 ppm. of calcium intensifies the yellow color of morin in alkaline solution. In 4 per ceut sodium hydroxide solution, the fluorescence due to lithium is 0.2 per cent of that of heryllium. Zinc fluorescence is perceptihle in daylight but is only ahout 0.005 per cent as intense as that of an equivalent amount of heryllium. Addition of potassium cyanide destroys the effect of the zinc, permitting detection of 1 part of heryllium in 20,000 parts of zinc. Cobaltic ion may be added to belp make the precipitation of scandium bydroxide more complete. Yttrium hydroxide may show a very faint fluorescence with morin.

If metals which form insoluble hydroxides are present, it is advisable to add excess alkali and examine under ultraviolet light. The precipitation of beryllium hydroxide may he diminished if aluminum is present in solution. Addition of tartrate or citrate ions to prevent the precipitation with hydroxyl-ion results in fluorescence due to any magnesium that may he present. Aluminum requires no separation since it does not interfere. The fluorescence varies in intensity inversely with sodium hydroxide concentration, necessitating close control of the alkalinity.

The method is only moderately accurate, the deviation being ahout ± 10 per cent for 0.0005-0.001 mg.

Procedure. Take an aliquot of sample containing 0.001-0.010 mg. of heryllium. Neutralize if acid, or too alkaline, and dilute or concentrate to ahout 4 ml. Unless the sample already contains ahout 0.4 per cent of sodium hydroxide, add sufficient to make the alkalinity equivalent to 1 ml. of 2 per cent sodinm hydroxide solution, and mix well. If sodium hydroxide has to he added, metals other than those which are amphoteric are precipitated as hydroxides. If a precipitate is obtained, filter, and wash well on the filter with small volumes of water.

At the same time set up a series of standards in similar volumes of water to which the same reagents have been added.

To sample and standards add 2 ml. of saturated sodium pyrophosphate solution. If zinc is present add 1 ml. of a 5 per cent solution of potassium cyanide. Add 0.1 ml. of a 0.02 per cent solution of morin in acetone, and adjust sample and standards to the same volume if necessary. For low beryllium content compare in ultraviolet light. A screened mercury glow lamp with a purple Corex glass shell is a suitable source of light. If more than 0.001 mg. is present, and the solution is free from opalescence and turbidity, add 0.4 ml. more of morin and make the comparison of the yellow color in bright daylight against a dark background. Compare without undue delay because the fluorescence slowly decreases with time or with exposure to sunlight.

Beryllium by Quinalizarin

When an alkaline solution of beryllium ions is treated with 1,2,5,8-hydroxyanthraquinone, quinalizarin, a blue lake is formed which may be measured colorimetrically.[12] The dye itself is violet in alkaline solution. Formation of a lake with aluminum is prevented by adding an excess of alkali. As little as 0.03 per cent of beryllium in aluminum can be determined by this reagent. Aluminum interferes in the presence of tartrate ions. Barium produces a similar blue of lower intensity.[13] Zinc gives a blue color. Metals precipitated by sodium hydroxide must be absent. Color due to copper and nickel is removed by addition of cyanide. The fluorescence has also been used as a measure of the beryllium content.[14]

After formation of the lake, addition of sodium hydroxide destroys color due to beryllium but not that due to magnesium. Analagously ammonium hydroxide destroys color due to the magnesium but not that due to beryllium. A convenient standard concentration of sodium hydroxide is 1 per cent unless much aluminum is present, when it should be raised to 2 per cent. In strongly alkaline solution the color of quinalizarin fades slowly

[12] Hellmut Fischer, *Z. anal. Chem.* 73, 45 (1922); *Wiss. Veroff. Siemens Konzern* 5, 99-119 (1926), *Z. anal. Chem.* 73, 54-61 (1928); Ichirô Iitaka, Yasuzô Aoki and Tomasada Yamanobe, *Bull. Inst. Phys. Chem. Research* (Tokyo) 14, 741-8 (1935); Giovanni Venturello, *Ricerca sci.* 14, 256-60 (1943).

[13] V. A. Nazarenko, *Zavodskaya Lab.* 4, 296-7 (1935).

[14] Mary H. Fletcher, Charles E. White, and Milton S. Sheftel, *Ind. Eng. Chem., Anal. Ed.* 18, 179-83 (1946).

Procedure. Take a volume of sample which will contain 0.001-0.01 mg. of beryllium and concentrate or dilute to 7 ml. or less. Neutralize any acidity or alkalinity present and add 1 ml. of 10 per cent sodium hydroxide solution. If necessary in order to dissolve aluminum increase to 2 ml. To a series of standards diluted to 7 ml. add the same amount of excess alkali that is used in the sample. Add 1 ml. of 0.01 per cent solution of quinalizarin in acetone to each and dilute to 10 ml. Mix and compare the colors at once.

Beryllium by Curcumin

In faintly alkaline solution a trace of beryllium is precipitated as the hydroxide and sorbs curcumin to give an orange-red color.[15] A solution containing 50 mg. of beryllium per liter gives a red flocculent precipitate. The method is suitable for quantitative estimation in concentrations from 0.05 to 1 mg. per liter. A blank solution is yellow to brown in color. Potassium, sodium, lithium, calcium, and barium do not interfere. Magnesium decreases the sensitivity of the reaction, although 1 mg. of beryllium may be detected in the presence of 1 gram of magnesium per liter. Aluminum and iron interfere but can be removed. Sodium fluoride decreases the sensitivity of the reaction.

Procedure. Measure out a suitable volume of sample solution, containing 0.0005-0.001 mg. of beryllium. To remove aluminum and iron acidify the solution slightly with 1:1 hydrochloric acid and treat with excess sodium fluoride solution. After 1 hour filter off the precipitate of sodium aluminofluoride, Na_3AlF_6. The amounts of iron and aluminum remaining in solution are not sufficient to interfere. Concentrate or dilute to 10 ml. and cool.

To the 10 ml. of sample and to 10 ml. portions of standard solutions, add 1 drop of a 0.1 per cent solution of curcumin in ethanol, 0.5 ml. of 20 per cent ammonium chloride solution, and 6-8 drops of 1:3 ammonium hydroxide. Standards and sample must be started simultaneously, as flocculation occurs on standing. Mix well and compare.

[15] I. M. Kolthoff, *J. Am. Chem. Soc.* **50**, 393-5 (1928).

CHAPTER 16

CHROMIUM

About 0.05 per cent of chromium is commonly present in igneous rocks. It is also frequently used in many yellow pigments, notably lead chromate and zinc chromate. Occurrence in tanned proteins adds other samples for analysis. Many samples are alloys, notably those of iron and steel. Since chromium forms a colored chromate ion, unlike aluminum and a number of similar ions, it can be determined without lake formation. The determination as adsorption compounds has therefore not been exploited to the same extent, although the use of lakes is as feasible as for aluminum.

The method by which chromium is oxidized and read as chromate or dichromate is suitable for relatively gross amounts. For lesser amounts the chromate is used to develop a color with a more sensitive reagent, thus using a form of indirect determination. The reagent is usually diphenylcarbazide. As described the samples are oxidized to chromate, since rarely is there occasion to have the final solution in any other form.

Samples

Air.[1] This method was developed particularly for analysis of the air in plating rooms doing chromium plating. Saturate filter paper with a solution containing 0.25 per cent *s*-diphenylcarbazide and 4 per cent of phthalic anhydride in 95 per cent ethanol, to 100 ml. of which 20 ml. of glycerol was added as humectant. Pass a known volume of the air through this paper and compare with permanent standards made with a solution containing equal amounts of methyl violet 2B and basic fuchsin. This method is that of pages 274-6 applied directly by this technic.

Nickel-chrome Alloys.[2] Dissolve a 1-gram sample in 5 ml. of 1:1 nitric acid and 20 ml. of 72 per cent perchloric acid. Evaporate nearly to dryness but do not bake the residue. Add 1 ml. more of perchloric

[1] Leslie Silverman and John F. Ege, Jr., *J. Ind. Hyg. Toxicol.* 29, 136-9 (1947).
[2] Winfield B. Sobers, *Am. Foundryman* 8, 45 8 (1945).

acid, dilute with water, filter, and dilute to a known volume for use of aliquots. The solution is also suitable for estimation of nickel, manganese, molybdenum, phosphorus, and silica.

Steel.[3] For steel containing up to 0.1 per cent of chromium dissolve 1 gram in 10 ml. of 1:1 nitric acid and 20 ml. of 70 per cent perchloric acid. For steel in the range 0.1-1.0 per cent of chromium reduce the sample to 0.5 gram and the perchloric acid to 15 ml. When dissolved evaporate to dense fumes of perchloric acid, and boil gently for 5-8 minutes to oxidize the chromium. Cool rapidly with tap water, take up in 20 ml. of water, and transfer to a 50-ml volumetric flask. Cool to room temperature and dilute to volume.

The chromium present as dichromate in this solution can be determined in the presence of colored ions such as copper, nickel, and cobalt, using the photometric method. Alternatively develop the color with 1,8-dihydroxynaphthalene-3,6-disulfonate and read at 525 mμ. For persulfate oxidation[4] dissolve 1 gram or less of sample in 15 ml. of 60 per cent perchloric acid and fume for 5 minutes. Let cool and add 30 ml. of a mixture of 100 ml. of concentrated sulfuric acid, 125 ml. of 85 per cent orthophosphoric acid, 250 ml of concentrated nitric acid and 525 ml. of water. Add 5 ml more of 85 per cent orthophosphoric acid and boil for 2-3 minutes Add 10 ml of 0 8 per cent silver nitrate solution and 140 ml. of hot water Heat to boiling and add 15 ml. of 25 per cent ammonium persulfate solution Boil for 1 5 minutes, transfer to a 500-ml. volumetric flask, and dilute to volume Chromium and manganese are present as chromate and permanganate ready for photometric reading. The ammonium persulfate oxidation may be followed by boiling with hydrochloric acid to reduce permanganate, and addition of fluoride to form a complex with ferric ion.[5] The iron may be precipitated with sodium hydroxide and sodium peroxide.[6]

There are numerous variations of these technics. Chromic ion may be oxidized to chromate by permanganate. In some the iron is separated.

[3] Louis Singer and Walter A Chambers, Jr., *Ind. Eng. Chem., Anal. Ed.* **16**, 507-9 (1944); Cf. Eugene H. Baker, *Foundry* **75**, 92,182 (1947).

[4] P. K. Kuchinskii and N. V. Kulmuikova, *Zavodskaya Lab* 1932, No. 7, 30 41: M. Misson, *Compt. rend. 17 me Congr. chim. ind.*, Paris Sept.-Oct. 1937, 111 12; R. W. Silverthorn and J. Alfred Curtis, *Metals and Alloys* **15**, 245-8 (1942); cf. I. V. Tananaev and K. A. Matveeva, *Zavodskaya Lab.* **11**, 615 (1945); M. Z. DeLippa, *Analyst* **71**, 34-7 (1946).

[5] H. Pinsl, *Arch. Eisenhüttenwes.* **10**, 139 43 (1936-7).

[6] W. Koch, *Techn. Mitt. Krupp, Forschungsber.* **2**, 37-46 (1938).

In these[7] high- or low-alloy irons are dissolved in acid, oxidized with permanganate, and the iron precipitated with sodium hydroxide. This necessarily involves risk of loss of chromate ion by sorption. As a typical technic allow for any acid already present, add 15 ml. of 1:1 sulfuric acid, and evaporate to a convenient volume. Add 10 ml. of concentrated nitric acid and boil until the fumes have disappeared. Add about 25 grams of ammonium phosphate, 250 ml. of water, and more nitric acid if ferric phosphate precipitates. Heat to boiling, add a saturated solution of potassium permanganate a few drops at a time until an excess is present, then 12 drops more, and continue boiling for 15 minutes.

Place 120 ml. of a 20 per cent solution of sodium hydroxide in a large beaker, add about 14 drops of a saturated solution of potassium permanganate, and boil for some minutes. Add more permanganate if the color changes to green. Add 10 ml. of a 5 per cent solution of manganese sulfate to destroy the permanganate. Pour in the acid solution of sample slowly. Transfer to a 500 ml. volumetric flask and let cool. Test to see that the solution is strongly alkaline. If not, add 4 per cent sodium hydroxide solution to distinct alkalinity. Then add 10 ml. of glacial acetic acid. Test to make sure that the solution is now acid. Dilute to volume, mix, and let settle. Filter through dry paper, rejecting the first 20 ml. This removes all colored ions except those of cobalt, nickel, and chromium. In the absence of nickel and cobalt the color should be nearly pure yellow. Dilute to a known volume for the use of aliquots.

Cast Iron.[8] Dissolve 0.1 gram in 10 ml. of 1:1 nitric acid. Dilute with enough water to avoid disintegration of the filter paper and filter off the graphite. Wash the residue with a minimal amount of water and add to the filtrate 10 ml. of a mixture of 1 part concentrated sulfuric acid, 1 part of 85 per cent orthophosphoric acid, and 8 parts of water. Add 5 ml. of 0.4 per cent silver nitrate solution and 5 ml. of 8 per cent ammonium persulfate solution. Heat to boiling for at least 3 minutes Chromium will be present as bichromate, manganese as permanganate. Remove the permanganate color by addition of a few drops of 95 per cent ethanol.

Brass. Dissolve 5 grams of brass in 50 ml. of 1:1 hydrochloric acid.

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[7] H. Pinsl, *Giesserei* 28, 429-34 (1941); cf. H. Ginsberg, *Metallwirtech. Metallwiss. Metalltech. Metallwirtschaft* 16, 1107-12 (1937).

[8] V. F. Mal'tsev and T. P. Temirenko, *Zavodskaya Lab.* 10, 357-61 (1941).

Nearly neutralize with saturated sodium carbonate solution, complete with a 10 per cent suspension of barium carbonate, and add 10 ml. excess of barium carbonate suspension. Boil gently for 10-15 minutes with a watch glass covering the flask to prevent oxidation of the iron. Filter rapidly and wash the precipitate twice with hot water. Transfer the filter paper and contents to a platinum crucible. Burn off the paper carefully and fuse the ash with a mixture of 5 grams of sodium carbonate and 0.25 gram of potassium nitrate. Dissolve the fusion in water, transfer to a beaker and add 2 ml. of 3 per cent hydrogen peroxide to reduce any permanganate formed. Boil a few minutes to decompose excess hydrogen peroxide and filter. Use as sample or dilute to a known volume and use an aliquot.

Iron Ore. Transfer a 1-gram sample to a nickel or iron crucible and mix with 10 grams of sodium peroxide. Fuse carefully and let cool. Take up the melt in water and filter the solution from suspended solids into a 250-ml. volumetric flask. Either read directly if the chromate gives sufficient color intensity or use an aliquot for determination by diphenylcarbazide. Before use of an aliquot by the latter method, boil for a few minutes to destroy the peroxide.

Minerals.[9] Mix 1 gram of 100-mesh sample with 4-5 grams of sodium carbonate and not over 0.5 gram of sodium nitrate in a platinum crucible. Cover and fuse for 20-30 minutes, longer if any remains undissolved as is apt to be the case with much chromite or magnetite present. After cooling take up with a little water to loosen the cake and transfer to a beaker. Heat with 30-40 ml. of water, to which has been added 2-5 drops of 95 per cent ethanol to reduce manganate. When disintegration is completed, filter on a fine-grain paper previously washed with 20 per cent sodium carbonate solution. Wash the residue with 4-5 portions of 5 ml. of 1 per cent sodium carbonate solution. Make the filtrate up to 100 ml. and use an aliquot according to the chromium content. This technic, in slightly modified form, can also be applied as a micromethod.[10] Use other portions of the filtrate for vanadium and molybdenum.

If over 0.01 per cent of chromic oxide is present the method as chromate is suitable, otherwise a more sensitive method such as that with diphenylcarbazide is desirable.

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[9] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* **8**, 336-41 (1936).

[10] B. I. Frid, *Zavodskaya Lab.* **11**, 17-23 (1945).

Soil.[11] Fuse 1 gram of soil in platinum with an excess of sodium carbonate. Take up the melt with a small volume of water and add 1:1 nitric acid until definitely acid. Heat to boiling and filter. Add 8 per cent sodium hydroxide to the filtrate until it is almost neutral and heat to boiling. Cool and pour the solution into a cold aqueous solution of 2 grams of sodium peroxide, keeping the mixture chilled. Heat on a water bath until the precipitate settles, let cool, and filter. Wash the precipitate well and reserve for determination of cobalt. Neutralize the filtrate with 1:10 sulfuric acid and make to a known volume for the use of aliquots.

Ash of Organic Matter.[12] Digest the ash with 10 ml. of 48 per cent hydrofluoric acid and 4 drops of concentrated sulfuric acid to volatilize silica. Evaporate to dryness and ignite. Fuse the residue with 6 grams of potassium pyrosulfate, then take up the melt in 150 ml. of 1:10 hydrochloric acid. Add 2 ml. of 5 per cent ferric chloride solution to provide a collector, and heat the solution nearly to boiling. Add 1:1 ammonium hydroxide until aluminum, chromium, and iron are completely precipitated and the solution is just alkaline to litmus. Filter, and wash the precipitate with a hot 2 per cent ammonium sulfate solution until the washings are chloride-free.

Dissolve the precipitated hydroxides in 80 ml. of 1:25 sulfuric acid. Add 0.5 ml. of 1:1 nitric acid, 1 ml. of 2.5 per cent silver nitrate solution, and 20 ml. of fresh 10 per cent ammonium persulfate solution. Boil for 10 minutes. When oxidation is complete, neutralize with 5 per cent sodium carbonate solution to precipitate iron and aluminum, and filter. Dilute the filtrate to 100 ml. for use of an aliquot as sample, preferably by the diphenylcarbazide method.

Fabric. Ash a sample of fabric of suitable size and fuse the ash for 4-5 minutes with 0.5 gram of equal parts of potassium chlorate and potassium carbonate Cool, dissolve in a small volume of water, and filter if necessary. Use as sample or dilute to a known volume and take an aliquot

Oxidation to Chromate. If the chromium is available as chromic ion, or if there is doubt as to a sample being fully oxidized to chromate, persulfate oxidation is a suitable technic.

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[11] Annie M. M. Davidson and R. L. Mitchell, *J. Soc. Chem. Ind.* 59, 232-5 (1940).

[12] C. F J. van der Walt and A. J. van der Merwe, *Analyst* 63, 809-11 (1938); A. M. M. Davidson and R. L. Mitchell, *J. Soc. Chem Ind.* 59, 232-5 (1940).

In the presence of nickel or cobalt, transfer the sample to a flask, heat to boiling, and add 4 per cent sodium hydroxide solution until a precipitate separates, avoiding a large excess. Cool, filter into a comparison tube, and complete as previously outlined starting with "To each add 20 ml. of 1:3 sulfuric acid. . . ."

In the Presence of Colored Ions.[17] Select an aliquot of the sample solution to contain 0.02-0.15 gram of chromium, and which may contain colored ions not reduced by ferrous sulfate. Depending on the history of the sample, make such additions of 1:1 hydrochloric acid or 4 per cent sodium hydroxide as are necessary to adjust to approximate neutrality. Transfer to a 50-ml. volumetric flask and add 10 ml. of 70 per cent perchloric acid. Dilute to volume. Ferric perchlorate gives no color.

Transfer a portion of the prepared solution to a photoelectric colorimeter and add 10-20 mg. of ferrous ammonium sulfate to reduce the dichromate. If silica is present, allow to settle for 1-2 minutes. Then set the instrument at zero with a filter transmitting in the range 410-480 mμ. This provides the blank for colored ions present. Discard this solution, rinse the cell well with the prepared sample, fill, and read. Subtract the first reading from the second. This is a measure of the bichromate present and may be applied directly to a calibration curve obtained with the same amounts of the same reagents but without the interfering ions. The intensity of color is affected by ferric perchlorate, which must therefore be provided in the standard, if present in the sample.

Chromium by Diphenylcarbazide

This qualitative reaction for chromium in solution, made strongly acid with acetic or hydrochloric acid,[18] has been modified for quantitative use.[19] The intense violet color is sensitive to 0.008 ppm. in dilute sulfuric acid. In the presence of acetic acid the sensitivity is reduced to about half that, 0.015 ppm.

The color develops instantly in a solution acidified with sulfuric acid and is stable for a long time. The most satisfactory acid is phthalic added as the crystalline anhydride.[20] The resulting color is much more

[17] Louis Singer and Walter A. Chambers, Jr., *Ind. Eng. Chem., Anal. Ed.* 16, 507-9 (1944).

[18] P. Cazeneuve, *Analyst* 25, 331 (1900).

[19] A. Moulin, *Bull. soc. chim. Paris* [3] 31, 295-6 (1904); George P. Rowland, Jr., *Ind. Eng. Chem., Anal. Ed.* 11, 442-5 (1939); V. F. Mal'tsev and T. P. Temirenko, *Zavodskaya Lab.* 10, 357-61 (1941).

[20] J. F. Ege, Jr., and Leslie Silverman, *Anal. Chem.* 19, 693-4 (1947).

stable than with other acids. Acidified molybdate interferes by giving a violet colloidal suspension. If no more than 1 mg. of molybdate per 100 ml. is present and the ratio of molybdate to chromium is less than 10:1 it may be neglected in obtaining results accurate to 1 per cent. Mercury, silver, copper, lead, and gold interfere to a moderate extent, cobalt and nickel slightly. This is especially true if the acidity of the solution is low. Iron does not if the color is read photometrically after proper filtration. Vanadium does not interfere if not in excess of the chromium and up to a 10:1 ratio does not introduce a serious error. The color developed with vanadium fades within 15 minutes unless the amount present is large. Vanadium is separated by extraction as the 8-hydroxyquinoline compound. High concentrations of dissolved salts affect the intensity of color developed. The color conforms to Beer's law. For photometric determination a proper filter is sextant green with a maximum transmission at about 520 mμ. Then the color due to ferric ion is completely eliminated. The maximum absorption is at 540 mμ. The color after removal of iron may be read with the Lovibond tintometer.[21] The reaction is also applicable in alkaline solution.[22]

Procedure. Take an aliquot of the sample solution containing 0.0005-0.015 mg. of chromium which should be present as chromate or bichromate. Unless the photometric method is to be used measure out a similar standard. Dilute to about 60 ml. and make approximately neutral to methyl orange with 8 per cent sodium hydroxide solution without adding indicator to the solution. This is done most conveniently by titration of another aliquot with an internal indicator. Thus the required amount can be added to the sample without manipulation.

If more vanadium than chromium is present, add 0.1 ml. of 2.5 per cent solution of 8-hydroxyquinoline in 1:8 acetic acid. Extract with three 2-ml. portions of chloroform and discard the extracts. Add another 0.1 ml. of 8-hydroxyquinoline solution and repeat the extractions. The last extract must be colorless. Filter the solution through a wet filter paper to remove suspended globules of chloroform. Rinse the funnel with a few ml. of cold water and use this to wash the filter paper. In the absence of interfering amounts of vanadium this paragraph is omitted.

Prepare a reagent by dissolving 4 grams of phthalic anhydride and 0.25 gram of *s*-diphenylcarbazide in 100 ml. of 95 per cent ethanol. Adjust the acidity of the approximately neutral solution by adding 3.3

[21] B. Bagshawe, *J. Soc. Chem. Ind* **57**, 260 5 (1938).
[22] W. Koch, *Arch Eisenhuttenw.* **12**, 69 80 (1939).

ml. of 1:5 sulfuric acid, and transfer to a 100 ml. volumetric flask. Add 1 ml. of the reagent, dilute to volume, and mix. Any manganese present as permanganate is reduced. If a moderate amount of vanadium is present it gives a brown color in place of violet. In that case wait about 15 minutes for the interfering color to fade before reading. Read at about 540 mμ.

Chromium by Disodium-1,8-Dihydroxynaphthalene-3,6-Disulfonate

Analysis for small amounts of chromium has been simplified by use of the above reagent.[23] The resultant color is pink to red and the reaction is so sensitive that 0.001 per cent of chromium can be determined in a 2-gram sample of steel. There is no advantage in using the method when more than 0.6 per cent of chromium is present in such a sample. Iron gives a greenish color with the reagent unless phosphoric acid is present. Vanadium gives a brown color and titanium a red. If a considerable quantity of vanadium is present this produces a brown color which is apt to obscure the results. If the vanadium present is less than the chromium a correction may be introduced. Subtract from the percentage of chromium shown, one-third of the percentage of vanadium, as shown by a separate analysis, in order to obtain the true percentage of chromium present. If the ratio of chromium to vanadium is high the error is negligible and may be disregarded. A correction is also introduced by using a similar amount of vanadium in the standard. Tungsten and molybdenum do not interfere. Titanium is removed in the determination. The average error is less than 1 per cent.

Procedure. Measure equivalent volumes of sample and standard to contain about 0.001-0.005 gram of chromium and adjust the acidity and reagents to be the same in each. The bulk of any iron in the original sample should have been removed. Unless the sample was prepared with perchloric acid add 10 ml. of 70 per cent perchloric acid to each and heat to strong fumes of perchloric acid. When cooled below 100° add 15 ml. of water, and boil until free of chlorine.

Add 2 ml. of 85 per cent orthophosphoric acid and 8 ml. of concentrated sulfuric acid to each and dilute to about 100 ml. This destroys any color of residual ferric ion. The final solution should not contain more than 20 per cent of sulfuric acid by volume. Add 2 ml. of a 1 per cent aqueous solution of disodium-1,8-dihydroxynaphthalene-3,6-

[23] P. Koenig, *Chem.-Ztg.* **35**, 277 (1911); F. Garratt, *J. Ind. Eng. Chem.* **5**, 298 (1913); Frank W. Scott, *Chemist-Analyst* **24**, 4-6 (1935); *ibid.* **25**, 63 (1936).

disulfonate. A pink to cherry red color develops and may be compared by dilution after 15 minutes. Only that method is applicable because of the peculiar character of the color developed and the danger of interference. A suitable final dilution is such that 1 ml. contains 0.001 gram of chromium.

Miscellaneous

The color of chromic ion may be used for its estimation by comparison with a standard of similar composition similarly treated.[24] Best results are obtained with 16-24 mg. of chromium in 50 ml. of 1:8 sulfuric acid. The interference of nickel and vanadium is corrected by having similar amounts in the standard. Thus as applied to iron and steel, transfer a 2-gram sample to a flask. In another flask take 2 grams of standard. To each add 35 ml. of 1:8 sulfuric acid. Insert a funnel in the neck of each flask and heat until solution is complete, adding water from time to time. Cool quickly and filter. Wash the carbon on the filter with small amounts of water, dilute the filtrates to 50 ml., and compare.

An analogous method is colorimetric comparison of filtered one-bath chrome liquors with a diluted stock liquor or with a spent liquor which has been analyzed for chromium.[25] The basicity Cr_2O_3/H_2SO_4, varies only from 0.87 before use to 0.75 after use, so that the method is reasonably accurate. The method is not applicable to 2-bath liquors because of the variable amount of reduced chromium salts present with dichromates. Reduction of all of the chromium to chromic chloride is not satisfactory because of difficulty in getting a clear color and because of the time required. When read photometrically as the chromic chloride or sulfate the peak of absorption is at 580 mμ[26]

Chromic ion is also determinable with benzidine.[27] The blue ring resulting is compared with a series of standards and is not adaptable to give very high accuracy.

Serichrome Blue R dyes wool to a bright crimson with a faint bluish tinge. Commercially, subsequent treatment with chromic acid converts this to a fast navy blue color. The treatment of wool dyed with Seri-

[24] E. Fogel'son, *Zavodskaya Lab.* **2**, No. 9, 33 6 (1933).

[25] J. T. Wood and D. J. Law, *J. Soc. Chem. Ind.* **29**, 398 (1910); *J. Am. Leather Chem. Assoc* **5**, 295-7 (1910); H. C. Holland, *J. Intern. Soc. Leather Trades' Chem.* **27**, 52-3 (1943).

[26] C. T. Kasline, *Ind. Eng. Chem., Anal. Ed.* **8**, 463 (1936).

[27] N. A Tananaev, *Z. anorg. allgem. Chem* **140**, 320-34 (1924); G. T. Mikhal'chishin, *Univ. état Kiev, Bull. sci., Rec. chim.* No. 3, 85-9 (1937).

chrome Blue R with very dilute chromic acid solutions develops a further bluish tint which may be used for quantitative estimation by comparison with a series of prepared standards.[28] The same blue color is developed with molybdic, tungstic, and vanadic acids, permanganates, and ferric ion. To avoid interference by the original red color, remove it with 0.5 per cent sodium carbonate solution, which does not remove the chromed color. Thus, add 40 ml. of water, 0.1 gram of sodium sulfate, and 0.02 gram of sulfuric acid to a flask. Add 2 grams of wool flock and shake to insure thorough wetting of the fibers. Add 20 ml. of a 0.5 per cent solution of Serichrome Blue R. Stir well and heat on a steam bath for 30 minutes. Filter on a Büchner funnel, wash until the washings show no acid, and dry. Add 0.1 gram of dyed wool to the acidified solution containing the dichromate derived from the sample. Heat on a steam bath for 30 minutes. Filter by suction and wash on the filter with hot 0.5 per cent sodium carbonate solution until only a blue color is left. Unless the quantity of chromium is so great as to exceed the maximum of the series of standards, the chromium content can be read off from the colored wool.

Chrome salts and chrome tanning preparations give a violet trioxalato complex which is read photometrically.[29] Beer's law applies at 0.05-1.3 ppm. and 420 mμ or at 0.05-1.7 ppm. at 560 mμ. Iron does not affect the readings at the latter level. To 25 ml. of sample containing 0.01-0.05 gram of chromium add 2 grams of oxalic acid and boil for 3 minutes. Cool and dilute to 50 ml. Read the transmittance at a suitable wave length.

The color developed by chromate oxidation of aniline hydrochloride is read photometrically.[30]

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[28] G. C. Spencer, *Ind. Eng. Chem., Anal. Ed.* 4, 245-6 (1932).

[29] E. R. Theis, E. J. Serfass, and Albert Clark, Jr., *J. Am. Leather Chem. Assoc.*, 41, 449-58 (1946).

[30] I. V. Tananaev and K. A. Matveeva, *Zavodskaya Lab.* 11, 615 (1945).

CHAPTER 17

IRON

IRON IS PRESENT to a greater or lesser amount in almost everything, whether organic or inorganic, and thus a great variety of samples has been prepared for its determination. Usually the total iron is desired, but a limited number of methods permit estimation of ferric ion in the presence of ferrous, or vice versa.

Many different methods of determination of iron have been developed, a majority by reaction with phenolic groups. Fortunately with many of them, interferences are not numerous. Selection of a method of determination is not simple. The oldest and best known is the one using thiocyanate. A considerable number of other reagents such as *o*-phenanthroline and α,α'-bipyridyl also form red complexes with iron and have their place. Determination as bromide or chloride in strong acid has real merit and has been adopted by the American Society for Testing Materials.

Although methods on which substantial work has not been done in the last decade have been eliminated, nevertheless a large number remain. They are tabulated in Table 3 with certain data regarding each one.

TABLE 3. METHODS FOR IRON

Reagent	*Color*	*Applicable Amount of Iron (mg.)*	*Wave Length for Reading Transmittance (mμ)*	*Page*
Thiocyanate	red	0.1-1.0	480, 500	307
Hydrochloric acid	yellow	0.01-1.0	370, 400	312
Hydrobromic acid	yellow	0.006 0.12	600	313
Bipyridine	red	0.01-0.12	520	314
o-Phenanthroline	red	0.02-0.25	490, 525	314
α, α'-Bipyridyl	red	0.005-0.12	520	316
Thioglycolic acid	reddish purple	0.02-0.2	540	319
Salicylic acid	red	0.15-3.0	530	321
Sulfosalicylic acid	yellow, red	0 02-0.5	430, 520	322
Ferron	green	0.005-0.2	...	324
Tiferron	red, blue	0.005-1.0	500	325
o-Nitrosophenol	green	0.001-0.05	..	326
Nitroso-R salt	green	0.00025 0 01	660	328
Salicylaldoxime	red	0 01 0.2	..	329
8-Hydroxyquinoline	green	0.005 0.1	470, 570	330
Potassium ferrocyanide	blue	0.001-0.1	620	331
Potassium ferricyanide	blue	...	..	332
Protocatechuic acid	red	0.01-1.0	500	333

cipitate containing the hydroxides was set aside (page 81) in the separation of copper. Dissolve the precipitate from the paper with hot 1:5 sulfuric acid, using a 200-ml. volumetric flask as receiver. When cool dilute to volume for use of aliquots in determination of iron and manganese.

Aluminum-copper-nickel-manganese-iron Alloys. The sample solution was prepared for copper determination (page 80). Use the thiocyanate method.

Copper Alloys.[9] The usual determination is on an electrolyte from which copper and lead have been deposited quantitatively. If necessary, a sample may be prepared solely for the determination of iron. This sample is also suitable for estimation of nickel.

For samples containing less than 0.5 per cent of tin and 0.01 per cent of silicon, dissolve 0.1 gram in 13 ml. of 1:1 nitric acid and heat until brown fumes are expelled. For alloys containing less than 1 per cent of tin, 0.01 per cent of silicon, and 0.1 per cent of lead, dissolve a 1-gram sample in 25 ml. of a mixture of 50 ml. of concentrated sulfuric acid, 125 ml. of water, and 35 ml. of concentrated nitric acid. Heat until all brown fumes cease. For alloys containing appreciable amounts of tin or silicon, dissolve a 1-gram sample in 15 ml. of a solution made by adding 20 ml. of 48 per cent hydrofluoric acid to 180 ml. of saturated boric acid solution and 13 ml. of 1:1 nitric acid. Heat until brown fumes cease to be evolved.

For alloys containing over 0.5 per cent of tin but under 0.01 per cent of silicon, first treat a 1-gram sample with 13 ml. of 1:1 nitric acid for solution and, when decomposition is complete, add 25 ml. of water. Boil gently for 2-3 minutes to coagulate metastannic acid, let stand at 60-75° until the precipitate settles, and filter. Wash the precipitate with hot 1:50 nitric acid and set the solution aside. Mix the paper and precipitate in the original beaker with 3 ml. of concentrated nitric acid and add 5 ml. of 70 per cent perchloric acid. Heat gently until the paper is destroyed and then heat to copious white fumes. Cool and add 10 ml. of concentrated hydrobromic acid. Evaporate to 1 ml. or less and, if not clear, add 10 ml. more of the acid and repeat the evaporation. Take to incipient dryness but do not bake. Heat with 2 ml. of 1:1 nitric acid and 5 ml. of water to dissolve the salts and add to the filtrate previously reserved.

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[9] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 337-41. American Society for Testing Materials, Philadelphia, Pa. (1946).

Dilute the solution of sample to about 150 ml., add 1-2 drops of 1:100 hydrochloric acid, and electrolyze at about 0.5 ampere per sq.dm. to remove copper and lead.[10] About 15 minutes before the electrolysis is ended, add 5 ml. of a filtered 10 per cent solution of sulfamic acid to destroy nitrous acid. At the end of the electrolysis remove the electrodes and rinse with water as usual. If the iron content is under 1 mg., evaporate to 140 ml. and use the entire solution as a sample by the thiocyanate method. If more than 1 mg. of iron is present dilute to a known volume and use an aliquot containing less than 0.1 mg. Add sufficient 1:1 nitric acid to the aliquot to total 10 ml.

Alternatively, to use the method of determination as the bromide, follow the details for lead- and tin-base alloys below, following the optional technic provided when more than 0.1 per cent of nickel is present.

Lead- and Tin-base Alloys.[11] To a 1-gram sample add 15 ml. of concentrated hydrobromic acid to which 10 per cent by volume of bromine has been added. Run an equal volume of the reagent as a blank. Heat gently, minimizing loss of bromine, until solution is complete. Then heat to boiling until nearly all the bromine is gone but minimizing the evaporation of hydrobromic acid.

If the sum of tin and antimony is more than 20 per cent, add 2 ml. of 70 per cent perchloric acid. Heat over a free flame with swirling until copious white fumes appear. This will expel the bulk of the arsenic, antimony, and tin. Cool and wash down the sides of the flask with 10 ml. of concentrated hydrobromic acid. Heat just to boiling to expel any remaining bromine and to dissolve salts. When tin plus antimony are under 20 per cent, omit this paragraph.

Brass.[12] Transfer a 1-gram sample to a Vycor beaker and add sequentially 15 ml. of water, 1 ml. of 48 per cent hydrofluoric acid, and 10 ml. of concentrated nitric acid. Let stand, covered, until solution is complete, and boil off the oxides of nitrogen. Rinse the lid with distilled water and dilute to about 135 ml. Add a drop of 1:100 hydrochloric acid and electrolyze at 2 amperes.[13] Use the copper and lead-free elec-

[10] For details of this electrolysis see page 82.

[11] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 348-50. American Society for Testing Materials, Philadelphia, Pa. (1946).

[12] William Goodman, *Anal. Chem.* 19, 141-2 (1947).

[13] For details of this electrolysis see page 82.

trolyte as a sample for determination of iron by *o*-phenanthroline. For determination as the chloride continue as follows.

Add 150 ml. of water and pass hydrogen sulfide through the solution until precipitation of heavy metals is complete. Filter, wash the sulfides on the filter with 1:20 hydrochloric acid saturated with hydrogen sulfide, and discard the precipitate. Add a suitable form of iron-free boiling-stone and boil until hydrogen sulfide is expelled.

If the alloy contains over 0.1 per cent of nickel, add 10 ml. of a 1 per cent solution of aluminum chloride hexahydrate, $AlCl_3 \cdot 6H_2O$, heat, and add bromine water dropwise until iron and hydrogen sulfide have been oxidized. Add 1:1 ammonium hydroxide until the yellow color disappears and 2 ml. in excess. Heat just to boiling, filter, and wash the precipitate once with water. To the paper in a beaker add 10 ml. of concentrated nitric acid, mix well, and add 5 ml. of 70 per cent perchloric acid. Boil gently until the paper is destroyed and evaporate to about 0.5 ml. If less than 0.1 per cent of nickel is present omit this paragraph.

For less than 0.1 per cent of nickel, evaporate the solution to about 25 ml. and add 5 ml. of 70 per cent perchloric acid and a few drops of bromine water to oxidize the iron. Evaporate to 0.5 ml. In either case the sample has now been concentrated to 0.5 ml. for determination as the chloride. Unless all bromides have been expelled results will be low. Take up the sample in constant-boiling hydrochloric acid for the procedure.

Tin-base Babbit.[14] To a 0.5-gram sample add 5 ml. of concentrated hydrochloric acid and 1 ml. of concentrated nitric acid. Evaporate to dryness, cool, and add 5 ml. of concentrated hydrochloric acid to the residue. Evaporate, cool, and dissolve the residue in 7 ml. of concentrated hydrochloric acid. Add water to about 60 ml. Add 4 grams of granular zinc and warm for a half hour to separate copper, antimony, and tin as a spongy residue. Filter and add 0.3 ml. of 30 per cent hydrogen peroxide. Boil, cool, and dilute to 100 ml. Use aliquots, usually 10 ml., for determination by sulfosalicylic acid.

Lead-base Babbitt. To a 0.5-gram sample add 3 ml. of 1:1 nitric acid and 5 ml. of concentrated hydrochloric acid. Boil until the residue is white and evaporate to a brown residue. Add 5 ml. of concentrated hydrochloric acid and evaporate again. Add 7 ml. of concentrated hydro-

[14] E. I. Fogel'son and I. V. Kalmykova, *Zavodskaya Lab.* 12, 973-4 (1946).

chloric acid and water to about 60 ml. Heat, if necessary, to obtain a pure white residue and cool again. Filter the lead chloride and wash with cold 1:8 hydrochloric acid. Complete by the previous method starting at "Add 4 grams of granular zinc. . . ."

Tantalum.[15] Transfer 1-2 grams of finely powdered tantalum to a platinum dish and add 20-30 ml. of 1:4 nitric acid. Warm on a hot plate and add 48 per cent hydrofluoric acid drop by drop until solution is complete, or nearly so.

If any residue remains add 5 ml. of concentrated sulfuric acid and heat until that acid is almost completely evaporated. Add 20 grams of potassium pyrophosphate and fuse over a free flame. When a clear melt is obtained, let cool and add 40 ml. of concentrated sulfuric acid.

If the original sample dissolved completely in nitric and hydrofluoric acids, add 40 ml. of concentrated sulfuric acid. Heat until sulfur trioxide fumes are evolved and let cool.

In either case, next add about 350 ml. of water and heat to boiling until a clear solution is obtained. Let cool and add 100 ml. of 40 per cent tartaric acid solution. Add 1:1 ammonium hydroxide until the solution is alkaline to litmus, then 5 ml. of a 0.5 per cent manganese chloride solution. Mix well, saturate with hydrogen sulfide, and let stand overnight for precipitation of sulfides. Filter the precipitate on a small, hardened filter. Wash with a 0.4 per cent solution of tartaric acid, made ammoniacal and partially saturated with hydrogen sulfide.

Dissolve the precipitate from the filter with a minimal volume of 1:4 hydrochloric acid and evaporate that solution to about 1 ml. Add 2 ml. of 3 per cent hydrogen peroxide and transfer to a 50-ml. volumetric flask. Dilute to volume and use an aliquot.

Metallic Tungsten or Tungstic Acid.[16] The small amount of iron present in tungsten can seriously affect the properties, the usual contents being below 0.01 per cent. Oxidize a 5-gram sample of powdered metallic tungsten to tungsten trioxide by heating in the air. Alternatively weigh out a 5-gram sample of commercial tungstic acid.

Dissolve the sample in about 50 ml. of 30% sodium carbonate solution in a 100-ml. Pyrex beaker and warm to about 90°. Insert a platinum anode about 3 × 3 cm. and a copper-foil cathode of similar size. Apply a current density of 10-20 amperes per sq. dm. for about 25 minutes. Remove the copper cathode and insert a new one. Repeat until deposi-

[15] P. Klinger, E. Stengel and H. Wirtz, *Metall. u. Erz.* 38, 124-7 (1941).
[16] M. L. Holt and Donald Swalheim, *Ind. Eng. Chem., Anal. Ed.* 11, 254-8 (1939).

tion of iron appears to have ceased, as evidenced by failure to obtain appreciable change in color. This will usually require 3-4 cathodes.

Place the electrodes with their deposits in a 100-ml. beaker and add 30 ml. of water, then 5 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid. Heat until solution is complete. Add 5 ml. more of each acid and evaporate to about 10 ml. Dilute to about 50 ml. and filter off the tungstic acid. Render the filtrate sufficiently ammoniacal to just redissolve the copper. The iron is precipitated but is contaminated by some sorbed copper. Warm to coagulate, filter, and wash well. Dissolve the precipitate in 5 ml. of 1:1 nitric acid, dilute to 45 ml., and use as sample. If sufficient copper carries over to interfere it will be necessary to carry out a second precipitation as the hydroxide.

Bismuth or Bismuth Oxide. Dissolve a sample of 10-30 grams in 50 ml. of 1:1 hydrochloric acid. Dilute to 250 ml., filtering if necessary. Add 1:1 ammonium hydroxide until only faintly acid to litmus. Precipitate the bismuth by saturating with hydrogen sulfide. Filter and wash the precipitate well with 1:500 hydrochloric acid. Boil off the hydrogen sulfide from the filtrate, concentrate if necessary, and dilute to a known volume for use of aliquots.

Mercury.[17] Treat 50 grams of mercury with 20 ml. of water and 60 ml. of concentrated nitric acid. The ensuing vigorous reaction can be expected to dissolve the sample within less than an hour. Heat the solution on a water bath for 30 minutes, then bring to a boil and remove from the flame. Add pyridine to the hot solution so long as precipitation of ferric oxide continues. Filter and wash the precipitate with water. Dissolve the precipitate in an appropriate volume of hot 1:10 sulfuric acid, dilute to a known volume, and take aliquots for determination.

Lead Carbonate, Lead Oxide and Pig Lead. A residue containing the iron has been separated in determination of copper (page 88). Dissolve this precipitate from the paper with 5 ml. of 1:1 hydrochloric acid. If considerable bismuth is present, nearly neutralize, and precipitate the bismuth with hydrogen sulfide. In that case, filter, boil off the hydrogen sulfide, cool, and dilute to 250 ml. Otherwise merely dilute to volume for use of aliquots. The precipitate of bismuth sulfide will not sorb appreciable amounts of iron.

[17] A. Castiglioni, *Z. anal. Chem.* **114**, 257-60 (1938).

Red Lead.[18] Separation of lead as the sulfate may give low results. Mix a 10-gram sample with 25 ml. of a cold saturated hydrazine hydrochloride solution. Add 50 ml. of concentrated hydrochloric acid and boil for 10 minutes. Add water to a volume of 400 ml., in which all but possibly a small spongy mass of lead will be soluble. Let crystallize overnight, decant, and filter. Evaporate the filtrate to 100 ml. and again let crystallize. Filter and precipitate iron by rendering the filtrate faintly ammoniacal. Filter the ferric hydroxide and dissolve in 5 ml. of 1:4 nitric acid. Dilute to volume in a 25-ml. volumetric flask and use an aliquot.

Iron Ore.[19] To determine the iron in such a sample careful aliquoting is essential. Dissolve a sample of 0.4 gram in 25 ml. of concentrated hydrochloric acid, adding more from time to time as may be necessary. Transfer the solution with any residual silica to a 1-liter volumetric flask and dilute to volume. Let settle and from this take an aliquot for analysis, exercising the greatest precautions as to accuracy. For the thioglycolic acid method, pipet out 10 ml. of this solution as sample. Add 1 per cent hydrazine hydrochloride solution until the yellow of ferric ion disappears and use as sample. For the α,α'-bipyridyl method,[20] accurately pipet 1 ml. into a 100-ml. volumetric flask as sample and start at the point of addition of the reagent but double the amount.

Unsintered Metal Carbides.[21] Oxidize a 0.5-gram sample by roasting in a platinum crucible and fuse with excess of sodium carbonate. Extract the melt with water and discard the aqueous solution which contains tungsten. Ignite the washed residue, add 0.5 gram of potassium acid sulfate, and fuse. Cool the melt, and dissolve in 150 ml. of 2:1 sulfuric acid. Make up to 500 ml. and use aliquots as samples. This is also suitable for estimation of nickel, cobalt, and titanium.

Commercial Salts and Oxides. Iron present in commercial salts and oxides is often in the form of scale derived from the equipment used in their manufacture. This is difficult to dissolve and to obtain a truly representative result may require a sample of substantial size. In general, dissolve such samples in 1:1 hydrochloric acid and heat for several

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[18] H. Heinrichs, *Z. angew. Chem.* 41, 450 3 (1928).
[19] J. P. Mehlig and M. J. Shepherd, Jr., *Chemist-Analyst* 35, 8-14 (1946).
[20] J. P. Mehlig and M. J. Shepherd, Jr., *ibid.* 36, 52-5 (1947).
[21] H. Cox, *Analyst* 69, 235-7 (1944).

tion of iron appears to have ceased, as evidenced by failure to obtain appreciable change in color. This will usually require 3-4 cathodes.

Place the electrodes with their deposits in a 100-ml. beaker and add 30 ml. of water, then 5 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid. Heat until solution is complete. Add 5 ml. more of each acid and evaporate to about 10 ml. Dilute to about 50 ml. and filter off the tungstic acid. Render the filtrate sufficiently ammoniacal to just redissolve the copper. The iron is precipitated but is contaminated by some sorbed copper. Warm to coagulate, filter, and wash well. Dissolve the precipitate in 5 ml. of 1:1 nitric acid, dilute to 45 ml., and use as sample. If sufficient copper carries over to interfere it will be necessary to carry out a second precipitation as the hydroxide.

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[17] A. Castiglioni, *Z. anal. Chem.* **114**, 257-60 (1938).

content; frequently 0.01-0.02 gram is sufficient. Moisten with 6 ml. of water and add 3-7 drops of concentrated sulfuric acid and 1 ml. of 60 per cent hydrofluoric acid. Mix well and heat on a steam bath. After evaporation nearly to dryness, transfer to a sand bath. After ignition to dryness, fuse with potassium pyrosulfate and let cool. Take up the melt in 1:100 sulfuric acid and heat if necessary to complete solution. Use as sample or dilute to a known volume and use an aliquot. Sulfosalicylic acid is a suitable reagent.

Alternatively[27] fuse a sample such as 0.1 gram with the minimum amount of sodium and potassium carbonates, let cool, and dissolve in water. Facilitate solution by dropwise addition of 1:1 hydrochloric acid. When solution is as complete as possible, filter if insoluble matter remains. If this residue is believed to contain iron, ash the paper, fuse with potassium bisulfate, and add to the main solution. Add 10 ml. of 1:1 hydrochloric acid, evaporate to dryness, and bake at 110°. Take up in 10 ml. of warm 1:10 hydrochloric acid and filter out the silica. Wash on the filter with 1:100 hydrochloric acid and discard the silica. Concentrate the filtrate for use as sample or dilute to a known volume and use an aliquot.

In general phosphates and fluorides cause the greatest interference.[28] Fluorides are largely volatilized as in the next method in a pyrosulfate fusion produced by fusion with potassium bisulfate. There is little that can be done to eliminate phosphates.

Cryolite.[29] Fuse a 1-gram sample with an excess of potassium bisulfate until a substantially clear melt is obtained. Take up in 25 ml. of 1:3 nitric acid and boil to dissolve any remaining oxides. Dilute to a known volume and use a suitable aliquot, taking into account that much aluminum is present. Sulfosalicylic acid is a suitable reagent.

Soluble Silicates. Dissolve a 1-gram sample in the minimum volume of water in a platinum dish. Add 10 ml. of 48 per cent hydrofluoric acid and 1 ml. of concentrated nitric acid. Evaporate to dryness. Take up in 10 ml. of 1:5 nitric acid or, if not completely soluble, re-treat with hydrofluoric acid. Dilute the solution to volume in a 25-ml. volumetric flask to take aliquots.

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[27] M. F. Chigrin, *ibid.* 6, 758 (1937).

[28] A. B. Shakhkeldian and M. Shkitov, *ibid.* 6, 1083-5 (1937).

[29] A. Jordy, *Aluminium* 21, 27-31 (1939); P. Urech, *Helv. Chim. Acta* 22, 322-30 (1939).

hours to be sure that all iron is in solution. Filter at this point if necessary.

If the iron is not fully oxidized, add a few drops of 3 per cent hydrogen peroxide. Add 1 ml. of 10 per cent aluminum sulfate solution, and precipitate the iron and aluminum with 1:1 ammonium hydroxide. Filter, wash, and dissolve the residue in 5 ml. of 1:1 hydrochloric acid. Dilute to 50 ml. and use a suitable aliquot as sample.

Corundum or alumina,[22] ground to 250-mesh, is fused either with borax and sodium carbonate,[23] or with potassium bisulfate, to give a solution suitable for analysis for iron.

Glass Sand.[24] Transfer a 1-gram sample to a platinum crucible. Add 1 ml. of 1:1 nitric acid and 5 ml. of 48 per cent hydrofluoric acid. Cover and heat on a hot plate. Decomposition will usually be completed within 1 hour. Remove the cover, rinse into the crucible with hot water, and evaporate the contents to dryness. Cool, add 10 ml. of 1:1 nitric acid, and evaporate to dryness. Repeat a third time. Fuse the residue with 2 grams of potassium bisulfate and let cool. Dissolve the melt in 100 ml. of hot water and acidify with 4 ml. of concentrated hydrochloric acid. Add 1:1 ammonium hydroxide until just alkaline to methyl red, precipitating iron and aluminum hydroxides. Filter and wash well with hot 1% ammonium nitrate solution. Dissolve the residue from the paper with 5 ml. of 1:1 nitric acid and dilute to volume in a 50-ml. volumetric flask for the use of aliquots.

Glass.[25] Weigh a 0.25-gram sample into a platinum crucible and fuse to a clear melt with the minimum amount of sodium carbonate. Dissolve the melt in water, assisting if necessary by dropwise addition of 1:1 hydrochloric acid. Add 2 ml. of 70 per cent perchloric acid and evaporate to copious white fumes of perchloric acid, and finally with water. Filter, wash the precipitated silica on the filter with 1:100 hydrochloric acid, and discard the filter. Evaporate the filtrate to a suitable volume, usually 25 ml., and use all or an aliquot.

Silicate Minerals.[26] Use a sample of a size corresponding to the iron

[22] J. Raffin, *Ann. chim. anal.* 25, 56-7 (1943).
[23] A. N. Miklashevskiĭ, *Zavodskaya Lab.* 6, 1209-13 (1937).
[24] Michel B. Vilensky, *J. Am. Ceram. Soc.* 19, 91-2 (1936).
[25] John H. Yoe and Robert T. Hall, *J. Am. Chem. Soc.* 59, 872-9 (1937).
[26] I. P. Alimarin and B. I. Frid, *Zavodskaya Lab.* 10, 252-3 (1941).

content; frequently 0.01-0.02 gram is sufficient. Moisten with 6 ml. of water and add 3-7 drops of concentrated sulfuric acid and 1 ml. of 60 per cent hydrofluoric acid. Mix well and heat on a steam bath. After evaporation nearly to dryness, transfer to a sand bath. After ignition to dryness, fuse with potassium pyrosulfate and let cool. Take up the melt in 1:100 sulfuric acid and heat if necessary to complete solution. Use as sample or dilute to a known volume and use an aliquot. Sulfosalicylic acid is a suitable reagent.

Alternatively[27] fuse a sample such as 0.1 gram with the minimum amount of sodium and potassium carbonates, let cool, and dissolve in water. Facilitate solution by dropwise addition of 1:1 hydrochloric acid. When solution is as complete as possible, filter if insoluble matter remains. If this residue is believed to contain iron, ash the paper, fuse with potassium bisulfate, and add to the main solution. Add 10 ml. of 1:1 hydrochloric acid, evaporate to dryness, and bake at 110°. Take up in 10 ml. of warm 1:10 hydrochloric acid and filter out the silica. Wash on the filter with 1:100 hydrochloric acid and discard the silica. Concentrate the filtrate for use as sample or dilute to a known volume and use an aliquot.

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Soluble Silicates. Dissolve a 1-gram sample in the minimum volume of water in a platinum dish. Add 10 ml. of 48 per cent hydrofluoric acid and 1 ml of concentrated nitric acid. Evaporate to dryness. Take up in 10 ml. of 1:5 nitric acid or, if not completely soluble, re-treat with hydrofluoric acid. Dilute the solution to volume in a 25-ml. volumetric flask to take aliquots.

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[27] M. F. Chigrin, *ibid.* 6, 758 (1937).

[28] A. B. Shakhkeldian and M. Shkitov, *ibid.* 6, 1083-5 (1937).

[29] A. Jordy, *Aluminium* 21, 27-31 (1939); P. Urech, *Helv. Chim. Acta* 22, 322-30 (1939).

Blast Furnace Slag.[30] *Total Iron.* Dissolve a 0.1-gram sample in 10 ml. of concentrated hydrochloric acid and transfer to a 100-ml. volumetric flask. Titrate the iron to the ferric form by dropwise addition of 1 per cent potassium permanganate solution. Dilute to volume and use an aliquot as sample.

Ferrous Iron. Transfer a 0.1-gram sample to a 100-ml. volumetric flask and displace the air with carbon dioxide. Add 10 ml. of concentrated hydrochloric acid and warm if necessary to complete solution. Dilute to volume and use a suitable aliquot for determination by the ferricyanide method.

Boiler Scale. A sample solution as prepared for aluminum (page 242) is suitable for determination of iron on another aliquot, with thioglycolic acid as the reagent.

Portland Cement. Dissolve 0.1 gram of cement in 5 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid. Dilute to about 30 ml. and filter into a 50-ml. volumetric flask. Wash on the filter with 1:10 hydrochloric acid and discard the residue. Dilute the filtrate to volume for use of aliquots.

Dental Enamel.[31] Digest a 0.1-gram sample with 2 ml. of concentrated nitric acid until the minerals are decomposed. Evaporate to dryness and ignite to decompose nitrates without fusion. Then add 5 ml. of concentrated hydrochloric acid and evaporate to dryness on a steam bath. Take up the residue in 0.5 ml. of 1:10 hydrochloric acid and dilute to 10 ml. for use as sample. Use the α,α'-bipyridyl method because of the phosphate content. An average value for humans is 0.0008 per cent.

Nickel Plating Baths.[32] In brief, precipitate with cupferron to separate from aluminum, copper, etc. Extract the iron from the cupferron precipitate with amyl acetate. In turn, extract the iron from the amyl acetate solution by shaking with 1:1 nitric acid. Reduce this sample with hydroxylamine hydrochloride and determine the iron with o-phenanthroline.

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[30] N. M. Miloslavskiĭ, E. G. Vavilova and I. Daĭkhes, *Novosti Tekhniki* 1939, No. 7, 14.

[31] Lewis L. Engel, *J. Dental Research* **14**, 273-6 (1934).

[32] Earl J. Serfass, W. S. Levine, G. Frederick Smith, and Frederick Duke, *Monthly Rev. Am. Electroplaters' Soc.* **33**, 1189-97 (1946).

Battery Acid. To a 10 ml. sample diluted to about 30 ml. add 0.6 per cent potassium permanganate solution until a faint color persists. As an alternative add a few mg. of potassium persulfate. Evaporate to a small volume and drive off sulfur trioxide fumes until the volume remaining is approximately 1 ml. Dilute to 45 ml. As standard mix 1 ml. of concentrated C.P. sulfuric acid with water and dilute to 45 ml. Use the thiocyanate method.

Zinc Galvanizing Baths.[33] Dissolve a 1-gram sample in 15-20 ml. of concentrated nitric acid. To precipitate lead and zinc as citrates dilute to about 100 ml., add 0.5-1 gram of citric acid, and carefully add 1:1 ammonium hydroxide until precipitation appears to be complete. Filter and discard the precipitate. Add more 1:1 ammonium hydroxide until the solution is slightly alkaline, then a few drops of litmus solution. Add a saturated solution of succinic acid until the litmus changes to a rose color and warm gently. Any hydroxides of residual zinc and lead not removed as basic citrates will dissolve. Filter the precipitate of basic succinates of aluminum and iron, wash with hot water, and ignite to oxides. Take up the ash in 5 ml. of 1:1 nitric acid and dilute to 50 ml. for use of aliquots.

Red Phosphorus.[34] To a 1-gram sample in a dry 250-ml. beaker add successively 75 ml. of carbon tetrachloride, 75 ml. of water, and 15 ml. of concentrated nitric acid. Prepare a solution of 5 ml. of bromine in 15 ml. of carbon tetrachloride and add this under a hood at a rate which will avoid violent reaction. This necessarily means dropwise, or nearly so, from a buret or suction-bulb pipet. Stir until the formation of yellow crystals ceases. These are $PBr_3 \cdot 2CCl_4$. As an air bath for evaporation, set the 250-ml. beaker in a 400-ml. beaker, with the lips divergent. Heat on a hot plate down to about 5-6 ml., at which level frothing and evolution of brown fumes will occur. If the liquid is colored, add a few crystals of potassium chlorate and heat until colorless.

Let the solution cool, take up with 100 ml. of water, and boil at a rate which will reduce the volume to about 30 ml. in 45 minutes. The phosphorus is now present as orthophosphoric acid. Transfer to a 100-ml. volumetric flask and dilute to about 70 ml. as a sample for development of color by o-phenanthroline.

[33] G. A. Panchenko, *Zavodskaya Lab.* 4, 231-3 (1935).

[34] J. A. Brabson, O. A. Schaeffer, Anthony Truchan, and LaVerne Deal, *Ind. Eng. Chem., Anal. Ed.* 18, 554-6 (1946).

If the original phosphorus had been treated with aluminum, add 4 ml. of 10 per cent hydroxylamine hydrochloride solution instead of only 1 ml. After 15 minutes add 1 ml. of 10 per cent citric acid solution. The balance of the procedure is normal.

Organic Iron in Soil.[35] Transfer a sample containing approximately 0.1 mg. of iron to a micro-Kjeldahl flask. Add 5 ml. of 1:10 sulfuric acid and heat to white fumes. Heat for a minute and add a few drops of 30 per cent hydrogen peroxide directly to the boiling acid. Repeat if necessary to get a clear digestate. Let cool, dilute to a known volume, and use an aliquot as sample.

Ferrous Iron in Soil.[36] Pass a 20-gram sample through a 2-mm. sieve and add about 900 ml. of boiling water. Continue to boil for 1 hour, transfer to a 1-liter volumetric flask, and cool quickly. Dilute to volume with cold air-free water. Displace air from the flask with carbon dioxide and stopper tightly. Let stand until insoluble matter has settled out, usually 1 day. Determine the ferrous iron by the ferricyanide method.

Water.[37] *Total Iron.* Evaporate 100 ml. of sample, or less, to dryness in a silica or porcelain dish. With waters containing silt it may often be necessary to use as little as 10 ml. of sample. If the water contains suspended matter, add 5-10 ml. of concentrated hydrochloric acid before evaporation. If the sample contains organic matter, ignite the residue gently but do not unduly prolong the ignition as the solubility of the residue can be impaired.

To the cooled dish, add 1 ml. of 1:3 hydrochloric acid. Warm on the water bath, adding distilled water from time to time. To complete oxidation, add 0.6 per cent potassium permanganate solution until a pink color persists for 5 minutes. Then transfer to a container for development of color. For waters low in organic matter, simplify the development by boiling a 50-ml. sample with 5 ml. of 1:1 nitric acid for 5 minutes, adding 3 drops of 0.6 per cent permanganate solution and letting cool. The official method is by a series of thiocyanate standards but the sulfosalicylic acid method is also applicable.[38] Methods shown earlier (pages 16, 91) give solutions suitable for estimation of iron.

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[35] Norman Ashwell Clark and Dale H. Sieling, *ibid.* 8, 256-7 (1936).
[36] O. Braadlie and H. Bergh, *Tids. Kjemi Bergvesen Met.* 2, 49-51 (1942).
[37] American Public Health Association, "Standard Methods of Water Analysis," Ninth Edition, pp. 53-5 (1946).
[38] Makhlis, *Novosti Tekhniki, Ser. Gorno-Rudnaya Prom.* 1936, No. 25, 2.

Alternatively,[39] for rapidity transfer a 30-ml. sample directly to a Nessler tube. Add 10 ml. of 1:1 hydrochloric acid and heat in a water bath at approximately the boiling point for 20 minutes. Let cool to room temperature and add an approximately 0.6 per cent solution of potassium permanganate drop by drop until a pink color persists for 1 minute. Dilute to 45 ml. as sample for color development. By reduction of the iron to the ferrous state, o-phenanthroline is a very suitable reagent.[40]

Ferrous Iron. Add 10 ml. of 1:5 sulfuric acid to 50 ml. of sample. The official method is by ferricyanide.

Boiler Water.[41] For less than 0.1 mg. per liter use 200 ml., otherwise 100 ml. Add 5 ml. of concentrated hydrochloric acid and 5 ml. of 3 per cent hydrogen peroxide per 100 ml. Boil and cool. Determine by the thiocyanate method using the extraction method but substituting amyl alcohol and amyl acetate as the medium.

Chalybeate Preparations. The methods for water are applicable to these iron preparations after suitable dilution.

Sea Water.[42] Collect the sample in well-seasoned bottles to avoid possible leaching of iron from the container. The analysis is desirably carried out while the water is fresh, as the growth of diatoms may remove most of the iron. Filter waters rich in plankton immediately on receiving. If there is a delay before analyzing, acidify the water with sulfuric acid before storing.

Measure 100 ml. of water into a 500-ml. flask. Add 6 ml. of concentrated sulfuric acid and evaporate to sulfur trioxide fumes. This removes chlorides, fluorides, nitrates, and nitrites, and destroys the organic complexes in which the iron is present. Let cool down to about 100° C. and carefully add 80 ml. of water. Heat the covered flask on a steam bath until alkaline-earth sulfates are dissolved. Add 0 6 per cent potassium permanganate solution, drop by drop, until the color persists. Two drops are normally sufficient. Add a few ml. of saturated bromine water and boil off excess bromine. The solution should be clear, except possibly for

39 Francis J. Hallinan, *Ind. Eng. Chem., Anal. Ed.* **15**, 510 12 (1943).

40 D. H. Caldwell, *J. Am. Water Works Assoc.* **38**, 727-30 (1946).

41 W. Teichert, *Ita* **17**, 135-52 (1946).

42 Thomas G. Thompson, Raymond W. Bremner and I. Marion Jamieson, *Ind. Eng. Chem., Anal. Ed.* **4**, 288-90 (1932); Thomas G. Thompson and Raymond W. Bremner, *J. conseil. intern, exploration mer* **10**, 33-8 (1935).

a deposit of white silicious material. When the solution has cooled, transfer it to a 100-ml. Nessler tube, dilute to 85 ml., and use for development of color.

Prepare a sodium sulfate solution for use in the standards by dissolving 450 grams of hydrated sodium sulfate in water to make 1 liter. This is approximately saturated at 20°. To this add 0.4 ml. of 50 per cent sodium hydroxide solution. Heat and stir to precipitate iron. Filter and neutralize with 1:1 sulfurc acid.

To 22 ml. of the sodium sulfate solution add 50 ml. of water and 5 ml. of concentrated sulfuric acid. This is equivalent to the sodium present in sea water in the form of various salts. As a series, add suitable amounts of iron in the range up to 0.20 mg. per liter and treat these in the same way as the sample. The usual method is by thiocyanate; Beer's law holds only over a very restricted range.

Alternatively,[43] by use of magnesium as collector, the iron is efficiently removed as the sulfide. Results will not necessarily agree with the previous method. Filter a 500-ml. sample through a Seitz filter. This removes suspended organic matter which will yield iron to the solution in subsequent treatment. This filtration gives lower results than use of paper, indicating greater efficiency. The main portion of iron in sea water is in solution. Fluoride remaining in the water does not interfere.

To prepare an ammonium sulfide reagent, pass hydrogen sulfide through a wash bottle and then a 120-cm. train packed with glass wool, then through concentrated ammonium hydroxide to approximate saturation. Test the reagent to insure absence of iron.

Add 5 ml. of this reagent to the filtered, 500-ml. sample and bring to a boil for a few minutes. A fine precipitate forms, entrains the ferrous sulfide, and settles quickly on standing. The sulfide reduces all iron to the ferrous condition. Failure to precipitate would be due to insufficient alkalinity and is correctable by addition of concentrated ammonium hydroxide, usually 5 ml., and reboiling. Filter the precipitate on paper in a Gooch crucible and wash. To insure the absence of iron, soak all filter paper in 1:1 hydrochloric acid, and wash carefully before use.

Dissolve the precipitate from the crucible and paper with 20 ml. of 1:3 hydrochloric acid and boil off the hydrogen sulfide. Add 2 ml. of bromine water to oxidize the iron and boil off the excess bromine. Check the bromine water to insure that it has not extracted iron from the glass bottle. When the solution has cooled, add 5 ml. of concentrated

[43] Norris W. Rakestraw, Henry E. Malmcke and Eliot F. Beach, *Ind. Eng. Chem., Anal. Ed.* 8, 136-8 (1936).

hydrochloric acid and make alkaline with concentrated ammonium hydroxide. Iron is precipitated with no more than a trace of magnesium. Filter, wash, and discard the filtrate. Dissolve from the paper with 20 ml. of 1:3 hydrochloric acid. Use a Nessler tube for development of color as receiver and dilute nearly to volume.

Fruit Juices, Wine, Beer, and Other Beverages. The iron in such liquids is combined organically[44] and therefore must be liberated by one or another method of ashing. Phosphates may interfere with estimation in the ash but the *a,a'*-bipyridyl method is suitable. A normal content in beer is below 1 mg. per liter but in cloudy beer as much as 13 mg. has been found.

As a general method,[45] weigh a 10-gram sample, or such lesser amount as will contain 0.04 mg. of iron, into a 30-ml. micro-Kjeldahl flask. Add a glass bead and evaporate almost to dryness. Trouble with frothing is minimized by adding 2 drops of caprylic alcohol. Add 1 ml. of concentrated nitric acid and heat gently to initiate the reaction. If applied to beer or ale, increase the nitric acid to 3 ml. After the initial violent reaction add 2 ml. of concentrated sulfuric acid and start digestion. At intervals add 3-4 drops of concentrated nitric acid to insure complete oxidation to a clear, straw-colored solution.

When digestion is complete let cool and destroy nitrosyl sulfuric acid. If considerable calcium is present add 1 ml. of 30 per cent hydrogen peroxide drop by drop to effectuate this. If calcium is low the nitrosyl sulfuric acid is hydrolyzed by adding 2-3 ml. of water, heating to white fumes, then repeating the hydrolysis. Finally cool and dilute the sample solution to about 20 ml. for use as sample.

Alternatively,[46] transfer a suitable sample to a 25 x 150 mm. Pyrex test tube Usually 2-5 ml. is sufficient. Add 1 ml. of concentrated sulfuric acid and 1 ml. of 70 per cent perchloric acid. Heat over a free flame to digest the organic matter, which should require not over 10 minutes. Let cool and either dilute to volume for taking an aliquot or use directly as sample, determining with *o*-phenanthroline.

As another suitable method,[47] take the filtrate from which copper, lead, and tin have been removed for determination (page 26). Evapo-

[44] G. Bode, *Wochschr. Brau.* **50**, 521 3 (1933).

[45] H. L. Roberts, C. L. Beardsley and L. V. Taylor, Jr., *Ind. Eng. Chem., Anal. Ed.* **12**, 365-7 (1940).

[46] L. G. Saywell and B. B. Cunningham, *ibid.* **9**, 67-9 (1937); L. G. Saywell. *Fruit Products J.* **16**, 201, 214 (1937).

[47] W. S. Hubbard, *J Assoc. Official Agr. Chem.* **19**, 389 93 (1936).

rate this filtrate to about 100 ml. Add 10 ml. of 50 per cent ammonium acetate solution and 0.5 ml. of glacial acetic acid. Let stand for precipitation of ferric phosphate, and filter. Wash the precipitate on the filter with 1:50 acetic acid and discard the filtrate. Dissolve the precipitate from the filter with 5 ml. of 1:1 hydrochloric acid and dilute the solution to a known volume for use of an aliquot. The ferrocyanide method is suitable. If dry ashing is applied to wine, addition of a calcium or barium salt will prevent the formation of pyrophosphate.[48]

Biological Samples.[49] Comparison of dry ashing with sodium carbonate or calcium carbonate and wet ashing with sulfuric acid led to this wet-ashing method as giving 100 per cent recoveries. Trouble in the others is believed due to volatility of ferric chloride.

Transfer a sample containing at least 0.02 mg. of iron to a Kjeldahl flask and, if dry, add a few ml. of water. Add 5 ml. of concentrated nitric acid and 1 ml. of concentrated sulfuric acid. Heat just to boiling and add 5-ml. portions of concentrated nitric acid as the material chars until the oxidation is practically complete. Add 1 ml. of 70 per cent perchlorio acid and heat to white fumes. If colorless, cease at this point, otherwise continue to heat until no further change occurs. Let cool and dilute to about 25 ml. If calcium sulfate has precipitated, as from some milk samples, filter and wash the paper well before discarding.

Add a drop of bromophenol blue indicator solution to the acid solution and neutralize with concentrated ammonium hydroxide. Usually about 5 ml. will be required. Add 3 ml. excess of ammonium hydroxide and saturate the solution with hydrogen sulfide. Filter the precipitated sulfides on an inorganic filter hut do not suck the precipitate dry as some iron will be oxidized to soluble sulfate on air exposure. Discard the filtrate. Dissolve the precipitate, which may be merely a greenish coloration, in 1 ml. of 1:1 hydrochloric acid. Rinse the Kjeldahl flask into the filter with a similar volume of this acid and with several rinsings of water. Wash the solution through the filter with water and boil the filtrate and washings to remove hydrogen sulfide and dissolve colloidal sulfur. When cool, dilnte to a known volume for taking aliquots. If the degree of acidity is undesirably high, neutralize the excess with 25 per cent sodium hydroxide solntion before dilution to volume, avoiding enough to cause precipitation. This wet ashing avoids pyrophosphate formation hut more or less orthophosphate may be present. A similar

[48] Gh. Ghimicescu, *Mikrochemie* 22, 208-15 (1937).

[49] S. H. Jackson, *Ind. Eng. Chem., Anal. Ed.* 10, 302-4 (1938); cf. A. J. Woiwod, *Biochem. J.* 41, 39-41 (1947).

method is satisfactory with blood.[50] The α,α'-bipyridyl reagent is appropriate.

As a simpler form of wet ashing,[51] simply digest the sample with a mixture of 1 part of concentrated sulfuric acid and 3 parts of concentrated nitric acid, adding more nitric acid as necessary until reaction is complete. Let cool and add water. Evaporate to eliminate oxides of nitrogen derived from nitrosyl sulfuric acid and dilute to a known volume to take aliquots.

If the final iron is desired in reduced form[52] add 2 ml. of 2 per cent sodium sulfite solution to the digestate and heat until the solution is substantially colorless. Add a drop of 1 per cent alcoholic *p*-dinitrophenol solution and neutralize with 1:4 ammonium hydroxide. Render just acid and colorless with 0.5 *N* sulfuric acid and use as sample.

If large amounts of silica are present in ash from dry ashing it is essential that this be decomposed to liberate all the iron.[53] Treatment with hydrofluoric acid is not suitable in the case of plant ash. The presence of pyrophosphate in such an ash also gives low results.[54]

Non-hemin Iron in Tissue.[55] Grind 1 gram in a mortar with powdered glass, 5 ml. of saturated tetrasodium pyrophosphate solution, and 10 ml. of 10 per cent trichloroacetic acid. Transfer to a centrifuge tube and heat in a boiling water bath for 7 minutes. Centrifuge at once and decant. Wash the residue by dispersing in 2 ml. of saturated sodium pyrophosphate solution and 2 ml. of 10 per cent trichloroacetic acid. Centrifuge, decant, and wash again. A slight opalescence in the combined decantates will disappear on addition of ammonia. The usual determination is with *o*-phenanthroline or thioglycolic acid.

Hemin Iron. Subtract the value for non-hemin iron from total iron.

Blood. Practically every method of digestion for decomposition of

[50] Francis B. Shorland and Eunice M. Wall, *Biochem. J.* 30, 1047-8 (1936); Herbert I. Coombs, *ibid.* 30, 1588-91 (1936).

[51] A. De Niederhäusern and Enzo Ferrarini, *Boll. soc. ital. biol. sper.* 12, 229-30 (1937).

[52] Karl-Heinz Schaefer, *Biochem. Z.* 304, 417-24 (1940).

[53] Hale Cowling and Erwin J. Benne, *J. Assoc. Official Agr. Chem.* 25, 555-67 (1942).

[54] D R. Borgen and C. A. Elvehjem, *J. Biol. Chem.* 119, 725 34 (1937).

[55] Gerhard Brückmann and Samuel Geor Zondek, *ibid.* 135, 23-30 (1940).

organic matter is applied to blood. The following methods are representative.

Pipet[56] 0.2 ml. into a suitable tube. Add 1 ml. of concentrated sulfuric acid and heat to boiling for 3.5 minutes. When cool add 1 ml. of a saturated solution of potassium chlorate and heat to boiling for 3 minutes. When cool add 0.3 ml. of saturated potassium chlorate solution and boil for 2 minutes. When cool dilute to 10 ml. The sample may also be oxidized by boiling with a mixture of nitric and perchloric acids.[57]

As another technic[58] transfer a suitable sample such as 0.1 ml. to a tube and add 0.2 ml. of 1:1 sulfuric acid. Heat nearly to boiling and add concentrated nitric acid dropwise until the organic matter is decomposed. Heat to sulfur trioxide fumes and let cool. Add 1 ml. of water and again heat to sulfur trioxide fumes to decompose nitrosylsulfuric acid. Let cool and take up with water to use as sample.

As another alternative[59] digest the sample with excess of potassium permanganate in acid solution, remove excess with hydrogen peroxide and use. Addition of potassium persulfate will stabilize the iron in the ferric form.

One other technic[60] is to transfer 0.5 ml. of blood to a 50-ml. volumetric flask, add 2 ml. of concentrated sulfuric acid and mix well. Add 2 ml. of saturated potassium persulfate solution and shake. Heat on a water bath at 80° for 10 minutes to complete release of iron from protein.[61] Add water to about 25 ml., follow with 2 ml. of 10 per cent sodium tungstate solution and mix. Cool, dilute to volume, and mix well. Filter through a dry filter paper and take 20 ml. of filtrate as sample. As standard add 0.8 ml. of concentrated sulfuric acid, 0.8 ml. of saturated potassium sulfate solution and 0.8 ml. of 10 per cent sodium tungstate solution to distilled water in a comparison tube. Dilute to 20 ml. The accuracy of results by this method has been questioned.

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[56] Frederick Reis and H. H. Chakmakjian, *ibid.* 92, 59-63 (1931).

[57] Andree Drilhon and Marcel Drilhon, *Compt. rend. soc. biol.* 109, 1234-5 (1932); F. Nöthlich, *Intern. Rev. ges. Hydrobiol. Hydrog.* 36, 562 (1938); Eugenio E. Vonesch, *Anales. farm. bioquím.* (Buenos Aires) 10, 124-9 (1939).

[58] Herbert I. Coombs, *Biochem. J.* 30, 1588-91 (1936); B. Shorland and E. M. Wall, *ibid.* 30, 1047-8 (1936).

[59] Rosalie Brener and Walter E. Militzer, *J. Biol. Chem.* 125, 561-6 (1938).

[60] Sin Yin Wong, *ibid.* 77, 409-12 (1928); Jerome E. Andes and David W. Northup, *J. Lab. Clin. Med.* 24, 197-206 (1938).

[61] A. Appelsis Fabian, Adolph Sachs and Victor E. Levine, *Proc. Soc. Expl. Biol. Med.* 32, 662-4 (1935).

Serum, Plasma, or Spinal Fluid. The lesser amount of organic matter in these samples simplifies decomposition, the relatively stable hemoglobin is not a part of the problem.

Total Iron. Transfer a 2-ml. sample of blood serum[62] or plasma to a rimless test tube. Add 1 ml. of 1:30 hydrochloric acid, stopper, and incubate at 37° for an hour. Cool to room temperature and add 1 ml. of 20 per cent trichloroacetic acid. Mix well and let stand for 1 hour at room temperature. Cover the tube witb tinfoil and centrifuge for 15 minutes.

The clear upper layer is usable as sample in some methods, for others it needs an adjustment of pH. As one of these, use 4 volumes of the supernatant layer with 1 volume of saturated sodium acetate solution as sample. As another method of adjustment, add to a known volume of filtrate, 2 drops of 1 per cent alcoholic *p*-nitrophenol and neutralize with 1:4 ammonium hydroxide. Render just acid with 1:50 sulfuric acid and use. No error is introduced by traces of hemoglobin in the serum.

For dry ashing of serum measure the desired volume based on a normal iron content[63] of about 0.15 mg. per 100 ml. and evaporate to dryness Ash in an electric furnace over a period of about 8 hours. Treat the ash with a few drops of concentrated nitric acid and ignite again. Let cool and take up the residue in 1:1 hydrochloric acid.

Inorganic Iron.[64] Mix 10 ml. of plasma or serum with 10 ml. of 20 per cent trichloroacetic acid. Let stand for 20-30 minutes and filter. To 3 ml. or 6 ml. of filtrate add 1 ml. of concentrated sulfuric acid, mix well, cool, and dilute to 10 ml.

Cytochrome C.[65] Dissolve a 0.020-gram sample in water and dilute to 20 ml. Use 7 ml. as sample. Add 2 ml. of 0.57 per cent sodium hydroxide. Add 0.5 ml. of 30 per cent hydrogen peroxide, mix well, and let stand for about 3 hours to decolorize; overnight is preferable. Cover

[62] J. Lederer and A. de Maesschalck, *Arch. intern. méd. exptl.* 13, 385-93 (1938); J. Vonkennel and Th. Tillhig, *Klin. Wochschr.* 19, 117-81 (1940); Knud Broehner Mortensen and Carsten Olsen, *Compt. rend. trav. lab. Carlsberg, Sér chim.* 23, 235-48 (1940); Georg Barkan and Burnham S. Walker, *J. Biol. Chem.* 135, 37-42 (1940); Rubens Salomé Pereira, *Rev. brasil. biol.* 1, 271-7 (1941).

[63] A. Sachs, V. E. Levine, A. C. Andersen and A. Schmit, *J. Lab. Clin. Med.* 26, 734-9 (1941); J. J. Schenk, *Schweiz. med. Wochschr.* 74, 64-5 (1944).

[64] Julia Gil, *Arch. farm. y. bioquím Tucumán* 1, 101-15 (1943).

[65] David L. Drabkin, *J. Biol. Chem.* 140, 387-396 (1941).

sulfur trioxide fumes. Take up in water, dilute to a known volume, and use an aliquot as sample.

As an alternative, ashing and separation of the iron from heavy metals has been described under copper (page 106). Use an aliquot of the solution so prepared to contain 100-150 mg. of iron. Add 1:1 ammonium hydroxide dropwise until ferric hydroxide is precipitated, and 2 ml. in excess. Heat just below boiling for 1 hour to coagulate the precipitate, maintaining the ammonia by occasional additions. Finally filter and wash with 3 portions of water.

Discard the filtrate and dissolve the precipitate with two 1-ml. portions of 1:2 hydrochloric acid. Use a 50-ml. volumetric flask as receiver and wash the paper with an additional 1-ml. portion of acid. Wash the beaker and filter with water leading to a total of not over 25 ml. and use this as sample for development of color.

Organic Samples, Particularly Medicinals. The solution for this determination as described under lead has had copper, arsenic, lead, and zinc removed (page 26). Add 1.5 ml. of 1:2 hydrochloric acid, transfer to a separatory funnel, and shake with 5 ml. of toluene to remove dithizone. Discard the toluene layer after washing with water and use the acid solution for determination of iron.

Methods of Concentration. Iron may be extracted as the chloride by ether, concentrated by precipitation as the 8-hydroxyquinoline derivative at controlled pH, or collected by precipitation in the presence of manganese dioxide.

As the Chloride.[78] The sample should have been dissolved without use of sulfuric or phosphoric acid. If nitric acid has been used, evaporate to dryness and ignite at a low temperature to convert to oxide. Take up the sample in concentrated hydrochloric acid. Manual extraction of ferric chloride from hydrochloric acid solution in the range of 70-80 per cent of the usual concentrated acid can be practiced with ethyl ether but is tedious and rarely can more than 95 per cent of the iron present be removed. By mechanical intermittent extraction this should theoretically approach completion. Apparatus for the purpose is shown in Figure 18.

[78] S. E. Q. Ashley and W. M. Murray, Jr., *Ind. Eng. Chem., Anal. Ed.* 10, 362-3 (1938).

Dilute concentrated hydrochloric acid with one-tbird its volume of water and use for transfer of the sample to the extraction apparatus. Place 200 ml. of isopropyl etber in the flask with glass beads or silicon carhide chips to avoid bumping, and boil. The vapors condense and are delivered through the funnel to the bottom of the extraction flask. Because of photochemical reduction hy either etbyl ether or isopropyl ether it is necessary to carry out the extraction in the dark or in dim daylight. Otherwisc substantial amounts of ferric ion are reduced to ferrous and then are no longer extractable.

The droplets, in rising, extract ferric chloride, then flow over into the boiling flask. If the accumulation of iron in the latter flask interferes, as by foaming, remove and replace with fresh isopropyl ether. Usually only a minute amount of iron remains at the end of 8 bours, and in 16 hours removal is so complete that no qualitative test for ferric ion can be obtained.

Evaporate tbe ether from tbe extracted iron. Take up with 10 ml. of 1:1 hydrochloric acid, dilnte to a suitable volume, and use an aliqnot for development of color.

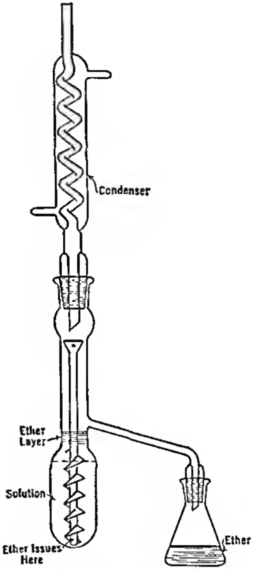

Fig. 18

Apparatus for Extraction of Iron. (Scientific Glass Apparatus Co., Bloomfield, N. J.)

By 8-Hydroxyquinoline.[79] Precipitation of iron, aluminum, and related metals with 8-hydroxyquinoline is a suitable method of concentration. Dilute or concentrate an aliquot containing 2-4 mg. of iron to about 100 ml. and neutralize with 1:1 hydrochloric acid or 1:1 ammonium hydroxide. Add 15 ml. of concentrated hydrochloric acid and dilute to about 150 ml. To this add 10 ml. of a 5 per cent solution of 8-hydroxy-

[79] R. O. Scott and R. L. Mitchell, *J. Soc. Chem. Ind.* 62, 4-8 (1943).

quinoline in 1:8 acetic acid. Add 1:1 ammonium hydroxide dropwise with stirring until the solution assumes an emerald green characteristic of the ferric complex at around pH 1.8. Then buffer the solution by adding 50 ml. of 15 per cent ammonium acetate solution which will raise the pH to about 5.1. Stir vigorously and let stand overnight. Filter and wash with cold water. Dry the precipitate and ash in a porcelain crucible at 450°. Dissolve this ash in acid of a type according to the method of development of color to be used, dilute to a known volume, and use a suitable aliquot for development of color.

Coagulation with Manganese Dioxide. Evaporate the sample solution nearly to dryness in fused silica to eliminate excessive amounts of acid. Take up in water, add 5 ml. of 1:5 hydrochloric acid, and dilute to about 100 ml. Add, with thorough mixing, 5 drops of 1 per cent potassium permanganate solution. Add 1:1 ammonium hydroxide until an excess is detectable by odor. Add 1 ml. of 95 per cent ethanol and mix. Heat just below boiling until the permanganate has all been reduced and precipitated as brown manganese dioxide. Filter and wash the precipitate on the filter. Dissolve the precipitate in 5 ml. of 1:5 hydrochloric acid and use as sample.

Standards

Ferric from Ferrous Ammonium Sulfate. Dissolve 0.7022 gram of crystallized ferrous ammonium sulfate, $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, in 100 ml. of water and add 10 ml. of 1:1 sulfuric acid. Warm and oxidize with approximately 0.1 per cent potassium permanganate solution until the iron solution remains faintly pink. Cool and dilute to 1 liter. One ml. corresponds to 0.1 mg. of ferric iron, or 0.143 mg. of ferric oxide.

Ferric from Ferric Ammonium Alum. Dissolve 0.6039 gram of ferric ammonium alum in water, add 10 ml. of 1:1 sulfuric acid, and dilute to 1 liter. Each ml. corresponds to 0.1 mg. of ferric oxide or to 0.07 mg. of iron.

Ferric from Iron Wire. Dissolve 0.1 gram of analytical-grade iron wire or powdered electrolytic iron [80] in 10 ml. of 1:10 sulfuric acid. Add 3 ml. of concentrated nitric acid and dilute to 1 liter. Each ml. contains 0.1 mg. of iron, or 0.143 mg. of ferric oxide.

[80] J. E. Lindsay, *Chemist-Analyst* **31**, a-9 (1942).

Ferrous from Ferrous Ammonium Sulfate. Dissolve 0.7022 gram of crystallized ferrous ammonium sulfate, $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, in freshly boiled and cooled distilled water containing 10 ml. of 1:5 sulfuric acid, and dilute to 1 liter. Each ml. corresponds to 0.1 mg. of ferrous iron. This must be freshly prepared.

Iron by Thiocyanate

The thiocyanate method depends on the oxidation of iron to the ferric condition after which the complex of ferric ion and thiocyanate ion produces a red color proportional to the amount of iron present.[81] The method is particularly useful for samples available in strongly acid solutions, thus simplifying adjustment of acidity, since optimum results are obtainable around pH 1-2, depending on the acid used. The method is so well recognized as to be standard with the American Society for Testing Materials,[82] Association of Official Agricultural Chemists,[83] and American Public Health Association. Although not the easiest to carry out or the most reliable it is probably the most widely used. The sensitivity compares favorably with many other methods used.

The intensity of color is increased by reduction of the dielectric constant of the solvent. For this the color may be extracted with a solvent of low polarity, such as ether or a higher alcohol, or the aqueous solution may be diluted directly.[84] For direct addition to the solution, acetone is commonly used. Addition of 2-methoxyethanol[85] is a desirable alternative[86] because of its low volatility. The color of the ferric thiocyanate can be stabilized by addition of ethylene glycol monobutyl ether.[87]

The usual extraction is with amyl alcohol-ethyl ether mixture. A variation in extraction technic is to use ether containing sulfur dioxide.[88] For the purpose saturate the ether with sulfur dioxide and for use mix 1

[81] H. Ossian, *Pharm. Centr.* 13, 205 (1837).

[82] Am. Soc. Testing Materials, "Standard Methods," Vol II, p. 685 (1939); "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 337 9, 354-5. American Society for Testing Materials, Philadelphia Pa. (1946).

[83] "Methods of the Association of Official Agricultural Chemists," Sixth Edition, p. 157 (1945).

[84] W. McKim Marriott and C. G. L. Wolf, *J. Biol. Chem.* 1, 451-61 (1905 6).

[85] The trade name is Methyl Cellosolve, Carbide & Carbon Chemicals Corp.

[86] H. W Winsor, *Ind. Eng. Chem., Anal. Ed.* 9, 453 5 (1937).

[87] Norris W Rakestraw, Henry E. Mahncke and Eliot F. Beach, *ibid.* 8, 136-8 (1936)

[88] K Steinhäuser and H. Olnsberg, *Z. anal. Chem.* 104, 385 90 (1936).

volume of this with 9 volumes of untreated ether. Increased stability of the extracted color is obtained.

As would be expected for so widely used a method, spectrophotometric absorption curves have been prepared for ferric thiocyanate and interfering ions.[89] The amount of color increases progressively with additional reagent, the peak of the absorption band shifting at the same time toward the red. The amount and kind of acid are interrelated. When hydrochloric acid is used with thiocyanic acid as reagent the optimum pH is 1.3 to 1.8. Use of ammonium thiocyanate as reagent raises the upper limit. Use of nitric acid and ammonium thiocyanate indicates the optimum to be below pH 1.0. Fading of the color is minimized by the presence of an oxidizing agent. By the use of nitric acid this is provided at the same time as pH control. At the optimum pH, increasing amounts of acetone up to 80 per cent by volume increase the sensitivity and greatly reduce the rate of fading in the light. The time of standing and amount of foreign ions in sample and standard must be carefully controlled.

Up to concentrations more than 250 times that of the iron, acetate, arsenate, benzoate, bromide, carbonate, chloride, citrate, cyanide, formate, nitrate, phosphate, salicylate, silicate, sulfate, and tartrate interfere to less than 2 per cent. The effect of metaphosphate and pyrophosphate can be overcome by addition of sufficient amounts of aluminum nitrate[90] but hydrolysis to orthophosphate and separation of the iron from phosphate appears preferable. The effect of phosphates is lessened by increase in acid concentration[91] and in thiocyanate concentration.

Ions which form colorless complexes with the iron interfere less in the presence of acetone. Thus less than 30 ppm. of fluoride are permissible in water but 400 ppm. in acetone can be tolerated. Pyrophosphate must not exceed 5 ppm. Oxalate must be completely absent in water, but 30 ppm. can be tolerated in acetone.

Iron is reduced by chlorostannite, iodide, nitrite, sulfite, and thiosulfate. Some bleaching occurs with arsenite, chlorostannate, and tetraborate. Vanadate and dichromate alter the hue and the latter oxidizes the reagent. Molybdenum on reduction forms colored complexes with thiocyanate. Tungsten interferes because of the low solubility of tungstic acid.

The color may be due to an anion, $Fe(CNS)_6^{\equiv}$ and in nonpolar

[89] J. T. Woods and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 13, 551-4 (1941).
[90] Henry E. Wirth, *ibid.* 14, 722-5 (1942).
[91] Charles A. Peters and Chester L. French, *ibid.* 13, 604-7 (1941).

solution to $Fe[Fe(CNS)_6]$ or to cations such as $Fe(CNS)^{++}$ and $Fe(CNS)_2{}^+$.[92] Varied filters have been used in obtaining the absorption, 470 mμ, 478 mμ, 494 mμ, even 553 mμ.[93] Apparently almost any filter in the blue-green is satisfactory. At 478 mμ Beer's law holds over the range 0.05-5.0 ppm. at pH 1.2-1.5 in 60 per cent acetone in a 1 cm. cell. When the color is extracted in amyl alcohol the filter may even have a dominant wave length around 660 mμ.[94] The relative instability of the color when exposed to light in absorption methods may give lower values than the *o*-phenanthroline method,[95] but in general the method agrees well with either that procedure[96] or the one using α,α'-bipyridyl.[97]

Persuasive arguments can be given for use of hydrogen peroxide as oxidant for the iron.[98] Thus no manganese is introduced to interfere, and the reduction in color due to sulfate is avoided. The bleaching of the color by light can be corrected by later addition of hydrogen peroxide. The bleaching of color is four times as great by sulfate ion as by chloride ion.[99] Powerful oxidizing agents produce yellow substances from thiocyanate.[100] In the case of hydrogen peroxide this is not difficult to control.

Results on traces of iron in silica[101] agree well with determinations by spectrum analysis.

Among limiting amounts which can be detected[102] are 0.000007 per cent in commercial sodium sulfate, 0.00002 per cent in water in the absence of hydrochloric acid and 0.000005 per cent in its presence,

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[92] Max Møller, *Kem. Maanedsblad* 18, 138 (1937); H. E. Bent and C. L. French, *J. Am. Chem. Soc.* 63, 568-72 (1941).

[93] A. D. Marenzi and E. Lida, *Anales farm. bioquím.* (Buenos Aires) 10, 12-16 (1939); P. Klinger, E. Stengel and H. Wirtz, *Metall u. Erze* 38, 124-7 (1941); H. Cox, *Analyst* 69, 235-7 (1944); D. G. Foulke and L. I. Horner, *Monthly Rev. Am. Electroplaters' Soc.* 32, 349-51 (1945).

[94] Kamenosuke Sinohara, *J. Biochem.* (Japan) 29, 57-79 (1939).

[95] Hale Cowling and Erwin J. Benne, *J. Assoc. Official Agr. Chem.* 25, 555-67 (1942); Erwin J. Benne and A. Joyce Snyder, *ibid.* 27, 526-31 (1944).

[96] L. G. Saywell and B. B. Cunningham, *Ind. Eng. Chem., Anal. Ed.* 9, 67-9 (1937).

[97] Philip P. Gray and Irwin M. Stone, *ibid.* 10, 415-17 (1938).

[98] Charles A. Peters, Majel M. McMasters and Chester L. French, *ibid.* 11, 502-3 (1939).

[99] A. Hedenstrom and E. Kanan, *Z. anal. Chem.* 91, 17-25 (1932).

[100] H. R. Offord, *Ind. Eng. Chem., Anal. Ed.* 7, 93 (1935).

[101] Heinrich Schlegel, *Angew. Chem.* 49, 411-12 (1936).

[102] H. W. van Urk, *Chem. Weekblad* 25, 703-6 (1928).

and 0.01 mg. per ml. in a solution of plant ash.[103] By micro technics on tissue amounts of 0.00005-0.0002 mg. are determined.[104]

A 20 per cent solution of pure ammonium thiocyanate is often used as reagent and is specified in the procedures which follow. If the color developed is too intense, repetition with a weaker thiocyanate solution will give less intense color from sample and standard. If both are too dark, they may be diluted to the same extent for easier comparison. While ammonium thiocyanate is specified throughout there is no reason why the corresponding sodium or potassium salts cannot be used.

Procedure. Transfer an amount of sample to contain 0.1-1.0 mg. of iron to a 100-ml. Nessler tube and dilute to about 90 ml. The solution should contain the equivalent of about 5 ml. of concentrated hydrochloric or nitric acid. Depending on the previous history of the sample solution, add acid or alkali to adjust to approximately that level of acidity. If there is any doubt about the iron being fully oxidized, add a few mg. of potassium persulfate or 1 ml. of 30 per cent hydrogen peroxide.

Balancing. Prepare a standard containing the same reagents as the sample, and a suitable amount of standard ferric ion solution. Dilute the standard to about 80 ml. To sample and standard add 5 ml. of 20 per cent ammonium thiocyanate solution. Add 2 ml. of ethylene glycol monobutyl ether to samples and standard before diluting to volume if it is desired that the color be stable for 1 hour, and dilute to 100 ml. Protect from light and compare as usual. If the color developed is not sufficiently intense repeat the preparation of sample but in dilution of sample and standard to volume use 50 ml. of acetone.

The color may also be intensified by extraction. For this take 25 ml. of the treated portions of sample and standard, in comparison tubes. To each add 5 ml. of a mixture of equal volumes of ether and amyl alcohol. Shake well and compare the colors of the upper layers by balancing. This procedure is particularly applicable for sample solutions containing less than 5 ppm. of iron. The developed sample to which this technic is applied must be one to which no acetone has been added.

Photometric. Omit preparation of the standard for balancing and

[103] W. Scholz, *Z. Pflanzenernähr., Düngung Bodenk.* 26A, 212-16 (1932).
[104] E. M. Scott, *Arch. Biochem.* 6, 27-32 (1945).

read the color in aqueous solution at 480 mμ, or at 500 mμ after extraction.

Duplication. Prepare a blank containing the same reagents as the sample, except for ferric ion. To blank and sample, add 5 ml. of 20 per cent ammonium thiocyanate solution. Dilute the sample to 100 ml. and the blank to about 95 ml. At this stage the blank should be colorless. Add to the blank a known solution of ferric ion, usually containing 0.1 mg. per ml., until the color of the standard so prepared matches that of the sample. When sample and standard are of the same color carefully adjust the volume of the standard to match that of the sample.

Dilution. Prepare sample and standard for balancing. Cautiously dilute the darker solution to obtain a match. The degree of dilution necessary should not be over 10 per cent.

Samples Containing Cobalt.[105] Even double precipitation of ferric hydroxide carries down substantial amounts of cobaltous hydroxide.[106] Electrodeposition carries along some iron. Precipitation with α-nitroso-β-naphthol or phenylthiohydantoic acid carries along iron more or less completely.[107] A single hydroxide precipitation can be used and the cobalt color filtered out photometrically.

After development of the thiocyanate color read with 425 and 525 mμ filters. Then solve by the equation

$$C_1 = \frac{0.281\, L^B - 0.178\, L^A}{25.07}$$

in which

C_1 = iron in mg. per 100 ml
L^A = reading by the 525 mμ filter.
L^B = reading by the 425 mμ filter.

This was developed with 5 ml. of 10 per cent ammonium thiocyanate and 5 ml. of concentrated hydrochloric acid per 100 ml. of developed solution.

Artificial Standards.[108] The color developed in 50 ml. by 5 ml. of 2 per cent potassium thiocyanate solution is approximated by this series

[105] Ernest A. Brown, *Ind. Eng. Chem., Anal. Ed.* 17, 228-30 (1945).
[106] G. E. F. Lundell and H. B. Knowles, *J. Am. Chem. Soc.* 45, 676-81 (1923).
[107] H. H. Willard and Dorothy Hall, *ibid.* 44, 2237-53 (1922).
[108] Daniel D. Jackson, *Technology Quarterly* (M. I. T.) 13, 320 (1900).

of standards, which however do not match the wave length distribution and, for careful work, must be checked against natural standards. As potassium chloroplatinate, K_2PtCl_6, dissolve 4 grams in distilled water, add 200 ml. of concentrated hydrochloric acid, and dilute to 1 liter with distilled water. As cobaltous chloride, $CoCl_2 \cdot 6H_2O$, dissolve 48 grams in distilled water, add 200 ml. of concentrated hydrochloric acid, and dilute to 1 liter with distilled water. It is essential that the cobaltous chloride contain the proper amount of water of crystallization. Place in 50-ml. Nessler tubes the volumes of platinum and cobalt solutions specified in Table 4 and fill the tubes up to the 50 ml. mark with distilled water. The iron equivalent is shown in the first column.

Other artificial standards can be prepared from mixtures of ferric chloride and cobalt nitrate in 1:20 hydrochloric acid.[109]

TABLE 4. PERMANENT STANDARDS FOR IRON BY THIOCYANATE

Mg. Iron	*No. of ml. of Platinum Solution*	*No. of ml. of Cobalt Solution*
0.0	0.0	0.0
0.01	1.00	0.60
0.02	2.25	1.20
0.03	3.30	1.85
0.04	4.65	2.75
0.05	5.75	3.65
0.075	8.85	6.60
0.10	11.30	10.00
0.125	14.70	12.80
0.15	16.85	15.10

IRON IN HYDROCHLORIC ACID

The yellow color of ferric chloride in concentrated hydrochloric acid has been adapted to the analysis of samples containing iron, when no interfering colors are present.[110] The color of ferric chloride is about as sensitive in the ultraviolet as that of the thiocyanate in the visible region. Over the pH region of 0-1 the extinction varies but little at 365 mμ. Usual amounts of other metals in aluminum do not interfere and 0.01 per cent of iron can be determined within 0.5 per

[109] T. D. Velichkovskaya, *J. Applied Chem.* (U.S.S.R.) 12, 1425-6 (1939).

[110] C. Hüttner, *Z. anorg. Chem.* 86, 341-57 (1914); J. C. Hostetter, *J. Am. Chem. Soc.* 41, 1531 (1919); "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 348-55. American Society for Testing Materials, Philadelphia, Pa. (1946).

cent. The amount of iron present must be less than 0.1 mg. per ml., and less than half of this concentration is better. The color intensity of ferric chloride reaches a maximum in 28 per cent hydrochloric acid and varies considerably with temperature. The presence of free chlorine does not interfere but nitric acid or its oxides do. Copper must be absent as its chloride has a color which interferes with that due to iron. Manganese does not interfere. A small amount of cobalt or nickel will not produce sufficient color to interfere. Zinc and mercury tend to bleach the color; calcium and magnesium intensify it. Sulfate ions decrease the color intensity. The peak absorption is at about 440-480 mμ.[111]

Procedure. The sample must have been dissolved in hydrochloric acid and been oxidized to the ferric condition. Bromine and bromides must be absent. Prepare constant boiling hydrochloric acid by dilution of 1 liter of concentrated acid with 900 ml. of water. If any yellow color is present distill off 150 ml. of the mixture and discard the distillate.

Transfer an aliquot of sample to contain 0.01-1 mg. of iron. If not in at least 1:1 hydrochloric acid, evaporate to a small volume. Take up with constant boiling hydrochloric acid, transfer to a 100-ml. volumetric flask, and dilute to volume with the same acid. Compare with a series of standards, with a single standard by balancing, by dilution with constant boiling acid, or by reading photometrically around 370 mμ. If the color is too intense read at about 400 mμ. If necessary to intensify the color by one of the comparison methods, add the same amount of calcium chloride to sample and standard solution.

A special standard solution in constant boiling hydrochloric acid is desirable. For this, heat 0.1000 gram of pure iron gently with 10 ml. of 1:1 hydrochloric acid. Add 1 ml. of 30 per cent hydrogen peroxide and boil until chlorine has been expelled. Cool and dilute to volume with constant boiling hydrochloric acid. The 0.1 mg. of iron per ml. is present in the ferric form.

Iron in Hydrobromic Acid

As the bromide in concentrated hydrobromic acid, copper and iron can be read photometrically at 460 mμ and 600 mμ respectively. Details have been given under copper (page 123).

[111] M. G. Mellon and C. T. Kasline, *Ind. Eng. Chem., Anal. Ed.* 7, 187-9 (1935).

Iron by Bipyridine

Ferrous iron forms a red complex with 2,2′-bipyridine, also known as α-α' bipyridyl, at pH 3.4. This is suitable for colorimetric estimation at 520 mμ.[112] Aside from the method given here for total iron, it is also used for estimation of ferrous iron for which a method is given separately. The desirable concentration is 0.02-0.24 mg. per 100 ml. The color remains stable for several hours, much longer if protected from oxidation. Copper in excess of 1 mg. in the solution may give a green complex and excessive amounts of zinc must be compensated by adding more reagent, as a colorless complex is formed with the reagent.

Procedure. Evaporate an aliquot of sample containing 0.01-0.12 mg. of iron, and preferably not over 20 mg. of aluminum, to dryness on a steam bath. Cool and take up with 2 ml. of 1:9 hydrochloric acid. Add successively about 15 ml. of water, 2 ml. of a 1 per cent solution of bipyridine, and 1 ml. of 10 per cent sodium sulfite solution. Mix and after 2 minutes add a further 2 ml. of the sodium sulfite solution. Transfer to a 50-ml. volumetric flask, dilute to volume, and mix. Filter if turbid, using paper if the iron is over 0.02 mg. but an inorganic filter to avoid sorption if below that level. Read the transmittance around 520 mμ and correct for a blank.

Iron by *o*-Phenanthroline

The orange to red complex formed between *o*-phenanthroline, also referred to as 1,10-phenanthroline, and ferrous iron[113] is a reversible, internal oxidation-reduction indicator,[114] which has been applied for colorimetric estimation The complex is the result of reaction of 1 mol of ferrous ion with 3 mols of the reagent, $[(C_{12}H_8N_2)_3Fe]X_2$. This is similar to the complex formed with α,α'-bipyridyl but the reagent is less expensive.[115] The color is proportional to the iron content at 0.1-6.0 ppm. over the pH range 2.0-9.0.[116] The method has the rather usual

[112] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 325-7. American Society for Testing Materials, Philadelphia, Pa. (1946).

[113] F. Blau, *Monatsh.* 19, 647-8 (1898).

[114] G. H. Walden, Jr., Louis P. Hammett and Ray P. Chapman, *J. Am. Chem. Soc* 53, 3908 (1931).

[115] G. Frederick Smith Chemical Co., Columbus, Ohio. *$1.75 gram.*

[116] L. G. Saywell and B. B. Cunningham, *Ind. Eng. Chem., Anal. Ed.* 9, 67-9 (1937); G. Frederick Smith and F. P. Richter, "Phenanthroline and Substituted Phenanthroline Indicators," G. Frederick Smith Chemical Co., Columbus, Ohio (1944); cf. H. G. Schmidt, *Biochem. Z.* **305**, 104-8 (1940).

variables in color development[117] depending on (1) order of addition of reagents, (2) time intervals between additions, (3) temperature of solutions, (4) presence of phosphates of varied types, and (5) period of standing before reading the color developed. The color becomes stable within 15 minutes, does not change for at least 48 hours thereafter,[118] and is suitable for either visual or photometric study. For measurement of transmittance use a filter centering around 490 mμ or 500 mμ.[119]

As the iron in solution is usually in the ferric form it is necessary to use a reducing agent. Various ones used have been hypophosphite, stannous chloride, and hydroxylamine hydrochloride. Sodium sulfite, sodium formate and formaldehyde form complexes with ferrous iron. Hyposulfite is unsatisfactory.[120] The most satisfactory reagents for this purpose are hydroquinone[121] and hydroxylamine.

Bismuth, zinc, and silver precipitate the reagent. Over 30 ppm. of antimonous ion causes precipitation of basic salts. Beryllium forms the hydroxide above pH 5.5, a complex below pH 3.0. By use of excess reagent 50 ppm. of cadmium, 10 ppm. of zinc, or 1 ppm. of mercury is permissible. Above pH 5.5 there is no interference by 100 ppm. of molybdate. Over 2 ppm. of nickel appreciable alters the hue. Tungsten as tungstate is tolerated up to 5 ppm. The pH must be above 6.0 to prevent interference by 500 ppm. of oxalate, above 3.0 for a similar amount of tartrate. Cyanide, in excess of 5 times the amount of iron, interferes. Nitrite does not interfere above pH 2.5. The pH must be above 6.0 to tolerate 50 ppm. of pyrophosphate. Interference by moderate amounts of pyrophosphate is best avoided by heating the sample for at least 15 minutes in 1:1 hydrochloric acid to hydrolyze to orthophosphate.[122] By reduction of the pH to 3-4, orthophosphate is precipitated as calcium phosphate from biological materials. Interference by thiosulfate is only that due to free sulfur deposited. Interference by chromic and cupric ions causing a change of hue is negligible below 20 ppm There is no interference with development of color by the presence of up to 500 ppm. of acetate, chloride, chlorate, bromide, iodide, nitrate, sulfate, sulfite, thiocyanate, citrate, arsenate, arsenite,

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[117] Selma L. Bandemer and P. J. Schaible, *ibid.* 16, 317-19 (1944).

[118] Michael Stevens Pepi, *Ind. Eng. Chem., Anal. Ed.* 18, 111-12 (1946).

[119] Karl Heinz Schaefer, *Biochem. Z.* 304, 417-24 (1940).

[120] A. Thiel, Hermann Heinrich and Eitelfriedrich van Hengel, *Ber.* 71B, 756-8 (1938)

[121] Frances C. Hummel and H. H. Willard, *Ind. Eng. Chem., Anal. Ed.* 10, 13-15 (1938).

[122] Hale Cowling and Erwin J. Benne, *J. Assoc. Official Agr. Chem.* 25, 555-67 (1942).

aluminum, barium, calcium, strontium, lead, manganous, magnesium, ammonium, potassium, sodium, and lithium.

A number of derivatives of the reagent have been studied. The main promise is shown by nitro 1,2-phenanthroline, which has the disadvantage of requiring 2 hours for full color development. The peak absorption band of the unnitrated compound is at 508 mμ with a secondary band at 474 mμ.[123] It requires approximately 1.2 ml. of 0.1 per cent reagent solution per ppm. of iron per 100 ml. The nitro compound is more purplish and lacks the band at 474 mμ.[124] A band is shown at 440 mμ by the 5-methyl compound but not by the 5-nitro-6 methyl derivative. Colors developed with *o*-phenanthroline are stable for 6 months and no advantage is shown by the derivatives. The method in some cases gives higher results than the thiocyanate method[125] but is more reliable.[126]

Procedure. Transfer the sample or pipet an aliquot containing 0.02-0.25 mg. of iron into a 100-ml. flask, and dilute to about 70 ml. Add 1 ml. of a colorless 10 per cent solution of hydroxylamine hydrochloride to insure complete reduction to ferrous iron. Let the solution stand for 15 minutes. Then add 10 ml. of a 0.25 per cent colorless aqueous solution of *o*-phenanthroline and mix well. Add sufficient 25 per cent sodium citrate solution to adjust the pH to approximately 3.5. The adjustment need not be accurate. The amount required will necessarily depend quite radically on the previous history of the sample. Dilute to volume, mix well, and let stand for 30 minutes at not less than 20°. Read either directly against standards similarly treated at the same time or by the transmittance with a filter of about 490 mμ, or 525 mμ.

If the sample contains substantial amounts of orthophosphoric acid modify the pH to which the solution is to be buffered. After addition of the reagent, adjust the pH to 6 ± 1 by dropwise addition of 1:1 ammonium hydroxide, determining the end point with test paper. At this pH allow 2 hours for color development.

Ferrous and Ferric Iron by α,α'-Bipyridyl or α,α',α''-Terpyridyl

Ferrous iron reacts with 3 mols of 2,2'-bipyridyl to form an intense

[123] W. B. Fortune and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 10, 60-4 (1938); J. P. Mehlig and H. R. Hulett, *ibid.* 14, 869-71 (1942).

[124] M. L. Moss, M. G. Mellon and G. Frederick Smith, *ibid.* 14, 931-3 (1942).

[125] Erwin J. Benne and A. Joyce Snyder, *J. Assoc. Official Agr. Chem.* 27, 526-31 (1944).

[126] Béla Bencze, *Mezögazdasátasok* 16, 61-9 (1943).

red complex $Fe[(C_{10}H_8N_2)_3]X_2$, in which X is any monovalent acid radical. The analogous reaction is given with 2 mols of 2,2′,2″-terpyridyl and is closely allied to that with *o*-phenanthroline. The color is suitable for colorimetric estimation.[127]

The technic can be manipulated to show amounts of the order of 1 part of iron per billion.[128] The color development is incomplete outside of pH 3-9 and that formed starts to fade at once below 2 or above 9.5. Determination at pH 4.0 will show 0.0002 mg. per ml.[129] Terpyridyl is usable in the range of pH 3-10. The maximum color absorption for the bipyridyl compound is around 522 mμ, for the ter-compound around 552 mμ. A 546 mμ filter is satisfactory.[130] As an alternative to usual methods of measurement of transmittance, the color can be read with Lovibond glasses.[131] Excess of reagent over that to produce the maximum color has no effect. The color developed with both the bi- and ter-compound follows Beer's law. For visual comparison the desirable range is 0.05-2 ppm.

The color reaction may be developed from ferric ion by addition of a suitable reducing agent. Titanous chloride, hydroquinone, hydroxylamine, ascorbic acid, *p*-hydroxyphenylglycine, sodium hyposulfite (hydrosulfite), sodium sulfite, and hydrazine sulfate have been used. The latter two are not very satisfactory. Colors developed with bipyridyl do not fade in 1 year when exposed to sunlight in Pyrex bottles. The ter-compound is stable for at least 3 months. When artificial standards are to be used, cobalt is suitable as having a similar wave distribution.

Uranyl ion alters the visible color but is screened out by measuring the transmittance at the optimum wave length. Orthophosphoric acid eliminates interference by benzoate, carbonate, formate, nitrite, oxalate, pyrophosphate, and silicate. Fluoride necessitates development of color for 45 minutes, 100 ppm. of tetraborate heating on a steam bath. Other

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[127] Fritz Blau, *Ber.* 21, 1077 (1888); *Monatsh.* 19, 647 (1898); Robert Hill, *Proc. Roy. Soc.* (London) B107, 205-14 (1930); J. P. Mehlig, *Ind. Eng. Chem., Anal. Ed.* 10, 136-9 (1938); S. H. Jackson, *ibid.* 10, 302-4 (1938); Elemer Schulek and István Flóderer, *Ber. ungar. pharm. Ges.* 15, 210-33 (1939); *Magyar Gyógyszerésztud. Társaság Ertesítője* 16, 210-1 (1940); Ruth A. Koenig and C. R. Johnson, *J. Biol. Chem.* 143, 159-63 (1942); Louis Gerber, Ralph I. Claassen and C. S. Boruff, *Ind. Eng. Chem., Anal. Ed.* 14, 364-6 (1942); J. P. Mehlig and M. J. Shepherd, Jr., *Chemist Analyst* 36, 52-5 (1947).

[128] L. H. N. Cooper, *Proc. Roy. Soc.* (London) B118, 419-38 (1935); Hans Borei, *Biochem. Z.* 314, 359-72 (1943).

[129] R. H. Thorp, *Biochem. J.* 35, 672-5 (1941).

[130] Kurt Buch, *Finska Kemistsamfundets Medd.* 51, 22-39 (1942).

[131] F. B. Shorland and E. M. Wall, *Biochem. J.* 30, 1047-8 (1936).

limits for negative ions are: 7 ppm. molybdate, tungstate; 10 ppm. cyanide; 50 ppm. vanadate; 100 ppm. chromate. Antimony, bismuth, and tin precipitate the reagent. The formation of complexes of other ions with the reagent cannot be corrected satisfactorily by excess of reagent and limits the amounts of such other ions as follows: 5 ppm. silver, copper, mercurous, zinc; 10 ppm. mercuric, nickel; 15 ppm. chromic; 20 ppm. cobalt, titanic; 50 ppm. beryllium, cadmium, zirconium; 75 ppm. manganous; 100 ppm. thallic.

By omission of the reducing agent, ferrous iron is determined in the presence of ferric.[132] α,α'-Bipyridyl appears preferable to 7-iodo-8-hydroxyquinoline-5-sulfonic acid because the latter is yellow, the additional green color in the presence of ferric ion does not follow Beer's law, and pH must be carefully controlled. The method is applicable directly[133] to degassed beer in which the iron is already in the ferrous form, by simple addition of the reagent and comparison with standards by the Walpole technic (Vol. 1, page 22). Nonhemoglobin iron in trichloroacetic-acid blood filtrates is so determined,[134] preferably by denaturing the plasma proteins with heat before precipitating.[135] The reaction is applicable to extracts of ionizable iron from foodstuffs[136] and to extracts of plant tissues.[137] Pyrophosphate interference is avoided by either fusing the sample with sodium carbonate or boiling it with hydrochloric acid.[138] When the iron content is insufficient to titrate with titanous chloride, the colorimetric method is a satisfactory alternative.[139]

Procedure. *Total Iron.* Measure an aliquot of sample to contain 0.005-0.12 mg. of iron but preferably not over 20 mg. of aluminum. Evaporate just to dryness on a steam bath, or with care on a hot plate. Cool and add 2 ml. of 1:15 hydrochloric acid. Then add 10-15 ml. of water and 2 ml. of a reagent containing 0.1 gram of α,α'-bipyridyl in 1 ml. of 1:15 hydrochloric acid and 9 ml. of water. Add 1 ml. of 10 per cent sodium sulfite solution, not over 3 days old, mix, and let stand for 2 minutes. Add 2 ml. more of the sodium sulfite solution and trans-

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132 W. J. Dyer and W. D. McFarlane, *Can. J. Research* 16B, No. 3, 91-6 (1938).

133 Philip P. Gray and Irwin M. Stone, *Ind. Eng. Chem., Anal. Ed.* 10, 415-17 (1938).

134 Eunice M. Wall and F. B. Shorland, *J. New Zealand Inst. Chem.* 1, 15-21 (1936).

135 C. A. Elvehjem and H. A. Schnette, *J. Biol. Chem.* 155, 653-60 (1944).

136 Leslie Shackleton and Robert I. McCance, *Biochem. J.* 30, 582 91 (1936).

137 W. E. Parker and F. P. Griffin, *Can. J. Research*, 17B, 66-70 (1939).

138 John S. Andrews and Clarence Felt, *Cereal Chem.* 18, 819-26 (1941).

139 G. E. Delory, *Analyst*, 68, 5-8 (1943).

fer to a 50-ml. volumetric flask. Dilute to volume and mix. Filter if turbid, preferably through a fritted glass crucible. Read the transmittance around 520 mμ and compare with a curve. For balancing, not over 2 ppm. of iron should be present. By transmittance greater latitude is possible by varying the thickness of cell.

Ferrous Iron.[140] Select an aliquot of sample containing 0.05-0.1 mg. of iron. Add 1 ml. of 1:10 sulfuric acid, 1 ml. of 1:4 orthophosphoric acid, 0.4 ml. of a 1 per cent solution of α,α'-bipyridyl, and 10 ml. of 20 per cent ammonium acetate solution. Dilute to 100 ml. and mix. Let this stand in the dark for 30 minutes for development of color and read the transmittance. The addition of orthophosphoric acid stabilizes the ratio of ferrous to ferric ion. A similar solution will give the value for total iron if 2-3 ml. of saturated sulfurous acid is added as reducing agent, and the whole is allowed to stand 24 hours for color development.

Artificial Standard.[141] A solution of cobalt nitrate containing 1.235 grams of the hexahydrate, $Co(NO_3)_2 \cdot 6H_2O$, per 100 ml. of water, or 2.5 mg. of cobalt per ml., is equivalent to ferrous bipyridyl containing 0.162 mg. per 100 ml. Such an artificial standard has an absorption curve so like that of the product of reaction that it can be used for the dilution or balancing methods.

Iron by Thioglycolic Acid

This reagent is variously described in the literature as mercaptoacetic acid, thioglycolic acid, thioethanoic acid, and thiolactic acid. It gives a blue to purple color with ferric ion, a red to purple with ferrous ion. So in practical use it reacts in ammoniacal solution to give a reddish purple color which is an equilibrium of the ferrous and ferric compounds.[142] The reaction is suitable for the spectrophotometer.[143] The color is stable for 12 hours if protected from light, is not affected by the concentration of reagent or pH within wide limits, and follows Beer's law.

The presence of 5000 ppm. of fluoride, iodide, nitrate, orthophosphate, sulfate, chlorate, tartrate, oxalate, citrate, acetate, bromide, thio-

[140] Elemer Schulek and István Flóderer, *Ber. ungar. pharm. Ges.* 15, 210-33 (1939); *Z. anal. Chem.* 117, 178-95 (1939).

[141] William D. McFarlane, *Ind. Eng. Chem., Anal. Ed.* 8, 124-6 (1936).

[142] Rudolph Andreasch, *Ber.* 12, 1391 (1879); Peter Claesson, *ibid.* 14, 412 (1881).

[143] H. W. Swank and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 10, 7-9 (1938); J. P. Mehlig and M. J. Shepherd, Jr., *Chemist-Analyst* 35, 8-14 (1946).

cyanate, sulfate, or chloride is without effect. Borate at 2500 ppm. does not affect the color. Pyrophosphate at 5000 ppm. reduces the color 8 per cent, at 2000 ppm. about 3 per cent. Cyanide must be absent. Nitrite gives a deep orange color to acid solution but not to alkaline. Up to 20 ppm. of molybdate does not affect the color of 1 ppm. of iron, more gives a yellow to orange color. More than 20 ppm. of tungsten as tungstate gives a blue color. No effect is shown by 500 ppm. of arsenic as arsenate.

Cobalt gives a yellow or red with about the same sensitivity as iron. Nickel reacts similarly but is less intense. Copper causes a bleaching of the iron color if present to over 10 ppm. More than 1000 ppm. of trivalent antimony will precipitate and a similar amount of trivalent arsenic bleaches the color. More than 200 ppm. of tin bleaches the color. Excess of reagent will prevent interference by 2000 ppm. of zinc or cadmium. Lead present must not exceed the iron content. By letting the solution stand a few minutes to decolorize, it will tolerate 100 ppm. of manganese. Other tolerances are magnesium 2500 ppm., bismuth 2 ppm., uranyl ion 0.2 ppm., gold 2 ppm. Mercurous ion gives a black precipitate but mercuric, sodium, potassium, and ammonium ions are tolerated. High salt content slightly decreases the color intensity. Aluminum or chromium are precipitated in the alkaline solutions used. Interference by pyrophosphate is avoided by boiling in strongly acid solution for 30 minutes. The amount of cobalt present as a decolorizer in clear glass is not sufficient to interfere[144] and thioglycolic acid is preferable to the thiocyanate method for glass analysis.[145] Iron must be separated from colored glasses.

Accuracy to 3 per cent or better is expected by varied methods. The balancing technic is suitable. Lovibond glasses are available to read the color produced in the presence of a citrate buffer. Photometric measurement around 535 mμ is suitable.[146]

Procedure. Measure out an aliquot of sample to contain 0.02-0.2 mg. of iron. Approximately neutralize, using an external indicator, and dilute to about 80 ml. If a standard is to be run in parallel, add the equivalent salts to it and dilute to the same volume. As reagent use 10 per cent thioglycolic acid solution, neutralized with 1:1 ammonium hydroxide to about the phenolphthalein end point. Add 2 ml. of the

[144] R. C. Chirniside and Celia F. Pritchard, *J. Soc. Glass Tech.* 23, 26-35T (1939).
[145] R. C. Chirniside, *ibid.* 22, 41-4T (1938).
[146] Ruth Adele Koenig and C. R. Johnson, *J. Biol. Chem.* 142, 233-8 (1942).

reagent and mix. Add 10 ml of 1:4 ammonium hydroxide, dilute to 100 ml., and mix. Compare or read photometrically at around 540 mμ.

Ferric Iron by Salicylic Acid

Ferric iron in reaction with salicylic acid produces an amethyst color suitable for colorimetric estimation, while ferrous iron produces no color.[147] The system follows Beer's law over a wide range of concentrations.

The formation of a ferric complex causes interference by tartrate, oxalate, citrate, orthophosphate, pyrophosphate, arsenate, cyanide, tungstate, and fluoride. Reduction of the ferric ion by sulfite, thiosulfate, and iodide interferes. Nitrite and thiocyanate alter the hue developed. Aluminum forms a colorless complex which interferes if present to over 10 mg. per 100 ml. Colored ions interfere. Removal of copper ion by cyanide is not satisfactory because cyanide ion is among those which interfere. The color is affected by pH and therefore the final solution is buffered. The color is stable for 48 hours in diffuse light but fades fairly rapidly in sunlight. The iron present should not exceed 10 ppm. in the final solution. Accurate comparison of less than 0.1 ppm. is impossible.

The method is applicable to solutions of iron ores containing 35-57 per cent of iron by titration, with an average accuracy of 0.20 per cent.[148] The degree of accuracy to be expected is about 2-3 per cent.

Procedure. Transfer a suitable aliquot of sample to contain 0.15-3.0 mg. of iron to a 100-ml. volumetric flask. If a standard is to be run in parallel, prepare it with similar contents of reagents.

If an equivalent acidity is not already present add 8 ml. of 1:1 hydrochloric acid. Add 1 ml. of a 10 per cent solution of sodium salicylate. The color will be amethyst. Add 10 ml. of 3 per cent ammonium acetate solution. The color becomes yellow. Now add 10 ml. of 1:1 acetic acid. The color returns to amethyst. The final pH is in the range of 2.5-2.8. Dilute to 100 ml. and compare with the standard or read the transmittance around 530 mμ.[149] The color is stable for over an hour.

[147] A. W. Gregory, *J. Chem. Soc. Trans.* **93**, 93-5 (1908); J. P. Mehlig, *Ind. Eng. Chem., Anal. Ed.* 10, 136-9 (1938); R. O. Scott, *Analyst* 66, 142-8 (1941); "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 343-4, 351-5; American Society for Testing Materials, Philadelphia, Pa. (1946).

[148] J. P. Mehlig, *Ind. Eng. Chem., Anal. Ed.* **9**, 162-3 (1937).

[149] Eugenio E. Vonesch, *Anales farm. bioquim.* (Buenos Aires) **11**, 1-7 (1940).

An alternative development of the color of the sample is to transfer to a 100-ml. volumetric flask and adjust the acidity to approximately that of 8 ml. of 1:1 hydrochloric acid. Dilute to about 50 ml. or more. Add 1 ml. of a 10 per cent solution of sodium salicylate to give the amethyst color. Add 1:1 ammonium hydroxide until the color is converted to yellow, and 0.2 ml. in addition. Add 1:1 acetic acid until the color is returned to amethyst, and 10 ml. in addition. Dilute to volume for comparison.

Ferrous and Ferric Iron by Sulfosalicylic Acid

Both ferrous and ferric iron form a yellow compound with sulfosalicylic acid, the 5-monosulfonate of salicylic acid, in ammoniacal solution.[150] The intensity of the color is independent of the concentration of ammonium hydroxide within fairly wide limits. In acid solution the same reagent gives a red compound with ferric ion, but the depth of color is influenced by the concentration of acid as well and therefore requires careful buffering of the solution. This is illustrated by the migration of the maximum with pH.[151] At pH 1.5 this maximum is at 500 mμ, at pH 5.0 it is at 460 mμ, and at pH 8.2 it is at 420 mμ.

The phosphorous content may be more than 100 times that of iron without interfering.[152] A moderate excess of ammonium salts is without effect. The presence of orthophosphate prevents interference by calcium and magnesium. Iron is separated from interfering amounts of manganese by precipitation with zinc oxide.

Strong oxidizing agents must be absent. The color of ions of copper, nickel, and cobalt interferes, but that of copper can be removed as the cyanide complex. Since the color in acid solution is only that of the ferric iron, and the color in alkaline solution shows all of the iron, the method is applicable to determination of both forms. Both colors are suitable for photometric determination[153] in the range 0.25-8.0 mg. ppm.[154]

The color in alkaline solution is most widely used.[155] Interference

[150] L. Lorber, *Biochem. Z.* **181**, 391 (1927).

[151] Martha Kennard and C. R. Johnson, *Proc. Trans. Texas Acad. Sci.* **27**, 45-51 (1944).

[152] L. C. E. Kniphorst, *Chem. Weekblad* **42**, 311-16 (1946); *ibid.* **42**, 328-34 (1946).

[153] N. M. Miloslavskii, E. G. Vavilova and I. Daikhes, *Novosti Tekhniki* **1939**, No. 7, 14.

[154] A. Thiel and O. Peter, *Z. anal. Chem.* **103**, 161-6 (1935); *cf.* A. Thiel and E. van Hengel, *Ber.* **70B**, 2491-7 (1937).

[155] V. M. Peshkova and A. D. Egorov, *Zavodskaya Lab.* **4**, 885-7 (1935).

by manganese can be successfully overcome by addition of hydroxylamine hydrochloride. Large amounts of aluminum or magnesium form complexes with the reagent. Results in their presence are greatly improved by addition of ammonium chloride[156] but usually addition of the interfering substance in the standard and an increase in the amount of reagent are necessary.[157] Sulfate ion interferes.

To determine iron in phosphatic materials such as apatite, omit addition of a citrate buffer, and increase the color-developing reagent by 5-fold. Under those conditions 1 part of iron can be determined in the presence of 100 parts of phosphate.[158] Unsatisfactory results are obtained in determination of iron in chromium plating baths[159] by both the thiocyanate and 7-iodo-8-hydroxyquinoline sulfonic acid or ferron methods but the sulfosalicylic acid method is satisfactory. When applied to borax and boric acid solutions, allowance must be made for the buffering effect of the sample.[160]

Procedure. *Total Iron.* Transfer an aliquot containing 0.02-0.5 mg. of iron to a 25-ml. flask. If the iron is not already in the ferric state, make acid and titrate with 1 per cent potassium permanganate solution to a faint pink color. Dilute to about 15 ml. If a parallel standard is to be run, prepare it in a similar flask, making equivalent additions of salts according to the previous history of the sample. Use a piece of Congo red paper as indicator. Neutralize with 10 per cent sodium hydroxide solution, then make just acid with 1:5 hydrochloric acid. Add 2 ml. of 10 per cent ammonium chloride solution, 2 ml. of 20 per cent sulfosalicylic acid solution, then 2 ml. of 1:10 ammonium hydroxide solution. Dilute to volume and compare, or read the color with a filter centering around 430 mμ.

Ferric Iron. If the sample has been so treated that the original ferrous iron has not been oxidized, take a sample similar to that used for total iron. If a standard is to be compared, prepare in a similar 25-ml. volumetric flask. To each add 0.1 *N* hydrochloric acid until acid to Congo red, and 0.1 ml excess, or the solution may already be acid. To each add 2 ml. of 10 per cent ammonium chloride solution, and 2 ml.

[156] E. I. Nikitina, *Zavodskaya Lab.* 9, 629-30 (1940).
[157] V. I. Kuznetsov, *ibid.* 12, 278-83 (1946).
[158] S. N. Rozanov, G. A. Markova and E. A. Fedotova, *Zavodskaya Lab.* 4, 639-48 (1935); *Z. Pflanzenernähr., Düngung u. Bodenk.* 41, 59-74 (1935).
[159] H. Pfeiffer, *Z. anal. Chem.* 126, 81-8 (1943).
[160] E. E. Zusser, *Zavodskaya Lab.* 8, No. 10-11, 1182-3 (1939).

of 20 per cent sulfosalicylic acid solution. Dilute to volume and compare. The transmittance may be read around 520 mμ.

Ferrous Iron. Subtract the ferric iron from the total iron.

Ferric Iron by Ferron

A saturated aqueous solution of ferron, 7-iodo-8-hydroxyquinoline-5-sulfonic acid, contains about 0.2 per cent of reagent and is yellow. The color is altered in acid solution by ferric ion to a green.[161] The reaction appears to be instantaneous. Ferrous ion gives no color. A slight greenish yellow distinguishable from the blank is given by 0.07 ppm., a distinct greenish yellow by 0.1 ppm., a bright bluish-green by 0.7 ppm., and green by increased concentrations. Ferron is a bright yellow crystalline solid. On standing several weeks a little liberation of iodine occurs in solution but the material is still satisfactory for reagent use. Three mols of reagent are required per mol of iron.

Citrate, cyanide, fluoride, orthophosphate, oxalate and tartrate interfere by forming complexes with the iron. Large amounts of aluminum, chromic, cobalt, nickel and uranyl ions react with the reagent to alter the color intensity but there is no interference by 5 times the amount of aluminum or by twice the amount of cobalt or nickel. Chromium should not exceed the iron and only a few tenths ppm. of titanium can be tolerated. Cupric ions to 0.2 ppm. interfere, fluoride causes partial bleaching, and stannous or nitrite ions may be present to no more than a fraction of a ppm. Interference by phosphate is avoided by standing for 2 hours. Beer's law is followed by the system. The color disappears completely below pH 1.2 and above 8.3. In the range of pH 2-3 the effect is slight, and once formed the color is stable over pH 2-5. No fading occurs in 36 days at pH 2.5. Even when exposed to diffuse daylight the color is stable for at least a week. There is no effect of temperature. Readily hydrolyzable salts interfere.

Because the color is affected by even small ranges of pH it is necessary to buffer the sample to 0.2 unit in the pH range 2-3. Citrate or phosphate buffers interfere with the reaction.

Only the series of standards and transmittance methods are suitable.[162]

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[161] John H. Yoe, *J. Am. Chem. Soc.* **54**, 4139-43 (1932); John H. Yoe and Robert T. Hall, *ibid.* **59**, 872-9 (1937); P. F. Hahn and G. H. Whipple, *Am. J. Med. Sci.* **191**, 24-42 (1936); Norman Ashwell Clark and Dale H. Sieling, *Ind. Eng. Chem., Anal. Ed.* **8**, 256-7 (1936); H. W. Swank and M. G. Mellon, *ibid.* **9**, 406-9 (1937).

[162] Yim-Chi Yin, *J. Chinese Chem. Soc.* **5**, 51-4 (1937); László Urbányi, *Mezõgazdasági Kutatósok* **15**, 265-70 (1942).

Procedure. Transfer an aliquot of sample containing 0.005-0.2 mg. of iron, and preferably 0.0075-0.05 mg. to a 50-ml. Nessler tube. Add 1:1 ammonium hydroxide to render just acid to Congo red paper. Then add 10 ml. of hydrochloric acid-potassium acid phthalate buffer for pH 2.6 (Vol. 1, page 173) and 1 ml. of saturated aqueous solution of the reagent. Compare with a series of standards or read the transmittance. For more than 2 ppm. of iron increase the reagent to 2 ml. Over 4 ppm. of iron cannot be read. The effect of excess reagent on the color can be eliminated with Corning HR filter 351.

Ferric Iron by Tiferron

The characteristic color development between *o*-dihydroxybenzene derivatives and ferric ion is accentuated in disodium-1,2-dihydroxybenzene-3,5-disulfonate,[163] which is known as tiferron. Below pH 5.0 the complex is deep blue. At 5.7-6.5 the color is violet, at 7.0 and above it becomes red. The reagent is sulfonated pyrocatechol. The red color is due to a complex of 1 mol of ferric ion and 3 mols of reagent, and the reaction is most sensitive with that reagent. Both the red and blue colors conform to Beer's law. The red color will detect 0.05 ppm. of iron, the blue about 0.3 ppm.

The reagent forms colored ions as follows: yellow with uranyl, titanic, molybdate, or osmate, greenish yellow with cupric, and purple with vanadyl fading in 15 minutes. Free perchloric acid in the sample darkens the color somewhat. Once the complex is formed, the solution may be made alkaline without ferric hydroxide precipitating. The usual complex-forming negative radicals interfere with the blue color but not with the red. For determination of iron in the presence of large concentrations of copper and nickel a modified technic is required.[164]

Procedure. Transfer an aliquot of sample containing 0.005-1 mg. of ferric iron to a 100-ml. volumetric flask. Dilute to volume and transfer 5 ml. to a Nessler cylinder. To similar cylinders transfer a series of graded amounts of standard ferric solution.

If the color is to be read in the blue region prepare a buffer solution for pH 4.0 containing 68 grams of sodium acetate trihydrate and 33.3 ml. of concentrated hydrochloric acid per liter. If the more sensitive red color is desired prepare a buffer for pH 9.5 containing 35.8 grams of

[163] John H. Yoe and A. Letcher Jones, *Ind. Eng. Chem., Anal. Ed.* 16, 111-15 (1944).

[164] R. H. Greenburg, *ibid.* 18, 255-7 (1946).

disodium phosphate dodecahydrate and 4 ml. of 4 per cent sodium hydroxide solution per liter. To each cylinder add 1 ml. of 0.25 per cent solution of the reagent and dilute to volume with the buffer. If precipitation occurs, let it settle before comparing.

Solutions High in Copper and Nickel. To the aliquot of sample in a 50-ml. volumetric flask add sufficient reagent solution to react with all the copper, nickel, and iron present. Each part by weight of iron requires 40 parts, copper 12 parts, nickel only 1.25 part. A large excess of reagent affects only the color due to nickel, and that only to a minor extent. Mix and dilute to volume with a buffer containing 2 per cent of sodium bicarbonate and 1 per cent of sodium carbonate. Mix well, and read the transmittance at 500 mμ within 4 minutes, with water as the blank.

To translate this reading it is necessary to have transmittance curves for the three metals as typified by those shown in Figure 19. These necessarily have to be obtained with the specific instrument and filter being used in the determination. Further the copper and nickel content of the sample must be known. As an example, an aliquot contained 12 mg. of copper, and the instrumental reading was 40. To correct, note that 12 mg. of copper correspond to a transmittance of 56. At 56 per cent transmittance the iron curve shows 0.074 mg. of iron, and at 40 per cent it shows 0.12 mg. The true value is therefore the apparent iron value 0.12, less the effect which the copper had on that value, 0.074, giving a corrected result of 0.046 mg.

Ferrous Iron by *o*-Nitrosophenol

The complex ion formed between *o*-nitrosophenol and ferrous ion is grass-green and suitable for colorimetric estimation.[165] The same reagent forms a red to violet complex with copper, mercury, and nickel. The technic includes extraction of the reagent from an organic solvent. Ferric ion forms a brown complex which is not quantitative and goes into the organic solvent layer. For determination of total iron the reduction to ferrous ion may use one of the usual reagents, such as isoascorbic acid. The method detects 0.01 ppm. and in its range of maximum sensitivity can be expected to be accurate to 0.5 per cent.

The system approximately follows Beer's law over the range concerned. Careful pH control at 5.1-5.3 is necessary. Complex-forming

[165] Georg Cronheim, *ibid.* 14, 445-7 (1942); Georg Cronheim and William Wick, *ibid.* 14, 447-8 (1942).

acids such as ortho- and pyrophosphoric, oxalic, etc., must be absent. There is no interference by ferric iron, cobalt, and palladium because their complexes dissolve in the organic solvent. More reagent is then needed. In strong light the reagent reduces ferric iron to ferrous, but not quantitatively. The other colored complexes with copper, nickel, mercury, and zinc can be screened out. Use of an acid medium avoids the color of the alkali salts of the reagent.

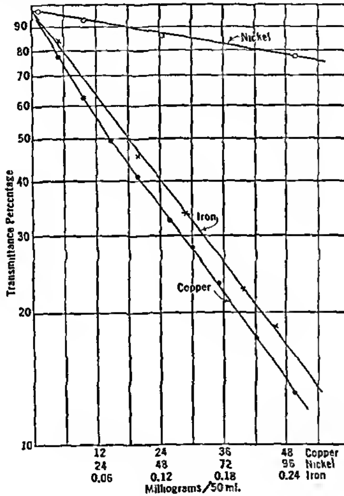

FIG. 19

Transmittance concentration Curves for Nickel, Iron, and Copper

Reagent.[166] Dissolve 2 grams of sodium pentacyano-ammine-ferroate in 100 ml. of distilled water. Ordinary ionized ferrous salts will not serve as a substitute. Add 25 ml. of benzene and 50 ml. of light petroleum ether. Cool the mixture with ice water. The color of the solution is brown. Dissolve 2 grams of hydroxylamine hydrochloride in the solu-

[166] Oskar Baudisch, *Science* 1940, 336; *J. Am. Chem. Soc.* 63, 622 (1941).

Procedure. Transfer an aliquot of sample containing 0.01-0.2 mg. of iron to a 100-ml. flask. Add 10 ml. of a fresh 0.1 per cent solution of salicylaldoxime in 5 per cent ethanol and mix well. Add 1 gram of solid ammonium acetate and mix well to dissolve. If in doubt as to full intensity of color having been developed add more reagent. Finally dilute to volume and compare.

The comparison may be with a series of standards, the color of which will be stable for at least 24 hours. It may be by dilution or balancing. If measured by transmittance, suitable filters are Corning Signal Blue No. 556 or Corning Light Blue Green No. 428.

Ferric Iron by 8-Hydroxyquinoline

The iron compound of 8-hydroxyquinoline has an intense dark green color in solution in organic solvents, which may be used for its colorimetric estimation.[169] While manganese, aluminum, copper, and zinc also react they are present in relatively small amounts in many samples and are not quantitatively precipitated at pH 5 under the conditions for precipitation of iron. The method is substantially to precipitate as the 8-hydroxyquinoline complex, separate, and dissolve in ethanol for comparison. Accuracy to 0.01 mg. or better is attained. With a certain amount of parallelism to the dithizone method, separations by extraction of the 8-hydroxyquinoline compound from mixtures have been investigated and offer promise as an alternative method.

The ferric compound can be extracted in the pH range 2.0-3.0.[170] Above the upper limit formation of the hydrous oxide interferes. The system conforms to Beer's law up to 20 mg. per liter of solvent, and the color may be read at either 470 mμ or 570 mμ. The reagent gives a measurable absorption. Both precipitation and extraction are closely dependent on pH. Around 470 mμ it is difficult to read even as high as 10 ppm. of ferric ion per liter. The pH for extraction would cause interference with copper, bismuth, and indium. At the optimum range for iron no appreciable aluminum is extracted to interfere, particularly near the lower limit for iron.[171]

Various derivatives of 8-hydroxyquinoline have also been investi-

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[169] J. Lavollay, 14 me Congr. chim. ind., Paris, Oct., 1934, *Bull soc. chim. biol.* 17, 432-8 (1935).

[170] Therald Moeller, *Ind. Eng. Chem., Anal. Ed.* 15, 346-9 (1943).

[171] J. W. Alexander, "Summaries of Doctrinal Dissertations, University of Wisconsin," Vol. 6, p. 205. University of Wisconsin Press, Madison, Wis. (1942).

gated.[172] Thus 8-hydroxyquinoline-5-sulfonic acid also gives a green color stable for 2 months. As with ferron, there are 3 mols required per mol of ferric ion. It is suitable for measurement of absorption at 580-610 mμ. In alkaline solution the color is altered to red. Oxalic and citric acids interfere by forming complexes with the iron, copper by forming one with the reagent. Phthalates and borates do not interfere and may be used as buffers. A somewhat similar dark green is given by 7-bromo-8-hydroxyquinoline-5-sulfonic acid and the 7-chloro compound. Ferron is the 7-iodo compound. Numerous other derivatives are less promising.

Procedure. Transfer an aliquot of sample solution containing 0.005-0.1 mg. of iron to a 35-40 ml. centrifuge tube. If the balancing method is to be used prepare an equivalent standard. Add 3 drops of glacial acetic acid, 3 drops of 0.2 per cent solution of methyl red in ethanol, and 1 ml. of 2 per cent sodium oxalate solution. This will keep the iron in solution in the presence of phosphates and precipitate calcium. Add 5 per cent sodium hydroxide solution until the indicator begins to change color. Add 2 ml. of a 2.5 per cent solution of 8-hydroxyquinoline in glacial acetic acid, then 1 per cent sodium hydroxide solution, drop by drop, until the indicator changes color. Iron hydroxyquinolate separates. Heat the tube in a boiling water bath for 10 minutes. Centrifuge while warm to separate the iron hydroxyquinolate and calcium oxalate. Decant the upper layer and wash the precipitate with water. Agoin centrifuge and decant.

Dissolve the precipitate in 95 per cent ethanol to which a drop or two of 1 per cent sodium hydroxide solution has been added, and dilute to 50 ml. or 100 ml. Compare or read the transmittance at 470 mμ or 570 mμ.

Ferric Iron by Potassium Ferrocyanide

The blue color of a colloidal dispersion of ferric ferrocyanide may be used for colorimetric determination.[173] The method is applicable in the presence of orthophosphoric acid. The presence of large amounts of copper or ferrous ion results in an interfering white precipitate. Large amounts of ammonium sulfate also interfere.[174] This method may be used

[172] Jacob Molland, *Tids. Kjemi Bergvesen* 19, 119-22 (1939); *Arch. Math. Naturvidenskab* 43, 67-184 (1940); *J. Am. Chem. Soc.* 62, 541-2 (1940).

[173] W. D. Walker, *Analyst* 50, 279-83 (1925).

[174] Sinitiro Baba, *J. Agr. Chem. Soc. Japan* 17, 139-43 (1941); *Bull. Agr. Chem. Soc. Japan* 17, 19 (1941).

to determine the ferric iron in the presence of reasonahle amounts of ferrous iron, or to determine total iron.

Accuracy to 1.5 per cent on qnantities as low as 0.01 mg. may he expected if the work is carefully done. Good agreement with the thiocyanate method has heen reported. The excess of potassium ferrocyanide introduces a yellow color which may be corrected hy ohserving the sample with artificial light transmitted through a yellow screen such as filter paper stained deep yellow with picric acid. The resulting effect is largely a matter of intensities of gray, the method heing more photometric than colorimetric. Gum ghatti or gum arahic solution can advantageously he added to render the suspension stahle. The transmittance may he read hy a 620 mμ filter.[175]

Procedure. Transfer an aliquot of sample containing 0.001-0.1 mg. of iron to a Nessler tnhe and dilute to ahout 20 ml. Prepare a series of standards with 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 ml. of standard iron solution containing 0.1 mg. of iron per ml. Each standard should contain the same volume of the same reagents as the sample. To each sample and standard add 1 ml. of a 1 per cent solution of potassium ferrocyanide and dilute to a uniform volume. Compare after 15 minutes. Permanent standards prepared with methylene hlne may also ba used. Alternatively read the transmittance at 620 mμ.

Ferrous Iron by Potassium Ferricyanide

Ferrous iron may he determined in the presence of ferric ion hy means of the deep hlue color of a colloidal dispersion of ferrous ferricyanide.[176]

Procedure. Prepare freshly hoiled and cooled distilled water. Transfer 75-ml. volumes to a series of Nessler tuhes. To each add 10 ml. of 1:5 sulfuric acid and suitahle volumes of the ferrous standard. Mix 50 ml. of the sample, after filtration to remove suspended matter if necessary, with 10 ml. of 1:5 sulfuric acid. To each standard and the sample add 15 ml. of freshly prepared 0.5 per cent solution of potassium ferricyanide in recently boiled and cooled distilled water. Dilute all to 100 ml. with the oxygen-free water and compare.

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[175] Eugenio E. Vonesch, *Anales. farm bioquim.* (Buenos Aires) **10**, 124-9 (1939).

[176] American Public Health Association, "Standard Methods of Water Analysis," Eighth Edition, p. 77 (1936).

Ferrous and Ferric Iron by Protocatechuic Acid

Another phenolic reaction with iron is that with 3,4-dihydroxybenzoic acid known as protocatechuic acid.[177] A blue-green color forms in solutions of ferric ion at around pH 5.0, under which conditions ferrous ion gives no color. The blue-green does not form in strongly acid solution and changes to red when the solution is made alkaline and ferrous iron also reacts. That color is destroyed if the solution is made too strongly alkaline.

The amount of reagent used should be held to a minimum. Of itself the reagent introduces a slight yellow color. A substantial excess causes the color to darken within 30 minutes; without an excess it is stable for an hour. On long standing the reagent oxidizes and the yellow becomes more pronounced. Temperature in the range of 20-30° has no effect. The red color conforms to Beer's law.

Copper interferes hy forming the ammonia complex but the method will tolerate 0.8 mg. of copper per 100 ml. Orthophosphate causes precipitation of calcium, if present, hut this can he prevented hy addition of citrate. The citrate ion has no effect on the color. Other ions slow down color development for as long as 5 minutes. The method is adapted to micro samples. To determine hoth ferrous and ferric ions do total iron in alkaline solution, ferrous iron in acid solution.

Procedure. *Total Iron.* Transfer an aliquot of sample containing 0.01-1 mg. of iron to a tuhe. If comparison is to he with a series of standards, transfer them to similar tuhes. If calcium and phosphate are present add 1 ml. of a 10 per cent solution of ammonium citrate. Develop the maximum color intensity hy addition of 0.05-0.2 ml. of a 1 per cent solution of protocatechuic acid in 50 per cent ethanol. Add, dropwise, a 25 per cent solution of ammonium sulfate in 1:15 ammonium hydroxide until the characteristic red color is developed. Dilute to a suitable volume, such as 10 ml., and compare. Alternatively read the transmittance of the sample with a 500 mμ filter.

Ferric Iron. Develop the color with the reagent in the presence of a huffer for pH 5.0 (Vol. 1, page 174).

Miscellaneous

The reaction with cupferron is adaptable to determination of iron

[177] O. Lutz, *Chem.-Ztg.* 31, 570 (1920); Rubens Salomé Pereira *J. Biol. Chem.* 137, 417-27 (1941); *Rev. brasil biol.* 3, 29-35 (1943); Rubens Salomé Pereira and Arnalo Costa, *Rev. faculdade med. vet., Univ. São Paulo (Brazil)* 2, 67-70 (1942).

in the presence of large amounts of phosphates.[178] Add sufficient sulfuric acid so that the sample contains 10 per cent by volume of the concentrated acid. The iron must all be in ferric form. Add 0.5 ml. of fresh 5 per cent cupferron solution and shake vigorously. Extract with 5, 2, and 2 ml. of chloroform, combine the extracts and dilute to 10 ml. Read the transmittance.

The red complex formed between 2,4-dihydroxybenzoic acid, β-resorcylic acid, and ferric ion is a phenolic reaction for estimation of iron.[179] For transmittance the minimum is in the range 425-450 mμ, the optimum pH at 2.5-3.0. The reaction is relatively insensitive, requiring about 20 ppm.

A *p-tert*-butyl-*o*-nitrosophenol reagent will react with ferrous iron.[180] To prepare the reagent add 0.4 gram of *p-tert*-butylphenol to 10-15 ml. of low boiling petroleum ether and warm until dissolved. Add this solution to 600 ml. of boiling water and evaporate the petroleum ether. Let cool and add 4 grams of hydroxylamine hydrochloride, and 0.5 gram of hydrated copper sulfate. Mix well, filter and add 2 ml. of 30 per cent hydrogen peroxide. The reagent is formed in 2-3 hours. Acidify the solution and extract with chloroform. Extract the chloroform with saturated lime water. Acidify and extract the lime water solution with petroleum ether. Use this petroleum ether solution to extract the iron from the sample, either that originally present as ferrous ion, or total iron after reduction with *d*-isoascorbic acid to ferrous ion. This is similar to a method with nitrosophenol given in greater detail (page 326).

Ferric iron gives a dark green color in faintly acid solution with pyrocatechol, *o*-dihydroxybenzene. If the concentration of iron is under 0.2 mg. per ml., the color is a brilliant violet, suitable for colorimetric estimation.[181] To 50 ml. of sample solution add 10 ml. of an aqueous 1 per cent solution of pyrocatechol. Shake and dilute to 100 ml. Compare with a fresh ferric solution treated similarly. An analagous reaction is given with a reagent consisting of saturated sodium sulfite solution containing 5 per cent of pyrogallol.[182]

Ferric iron forms a blue compound with pyramidone which is but little affected by acidity greater than 0.2 *N*.[183] The reaction will detect

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178 Robert Paulais, *Compt. rend.* **206**, 783-5 (1938).

179 Jean L. Larner and William T. Trout, Jr., *Virginia J. Sci.* **3**, 13 (1942).

180 Oskar Baudisch and George E. Heggen, *Arch. Biochem.* **1**, 239-45 (1942).

181 A. L. Bernouilli, *Helv. chim. Acta* **9**, 835 (1926).

182 A. P. Palkin, *Zavodskaya Lab.* **4**, 1106 (1935).

183 H. W. van Urk, *Pharm. Weekblad* **63**, 1121-3 (1926).

0.05 mg. of iron per 100 ml. To a 50-ml. sample add 0.5 ml. of 1:1 sulfuric acid and 10 ml. of a 10 per cent solution of pyramidone in 1:50 sulfuric acid. Dilute to 100 ml. with 1:50 sulfuric acid. Compare with a standard similarly treated.

Iron gives a blue color with alloxantin in alkaline solution.[184] Prepare the reagent by dissolving 0.1 gram of alloxantin in 10 ml. of 4 per cent sodium hydroxide solution. Destroy any color in the reagent by boiling and cool rapidly. To 2 ml. of ferric iron solution add 1 ml. of reagent. Compare with a standard similarly treated.

The red color produced by the reaction of ferrous iron with dimethylglyoxime is very distinctive and permits the estimation of the iron with an accuracy of 1 to 2 per cent.[185] Further study of dioximes [186] has also indicated the suitability of diethylaminobutanedionediaxime, butanedionedioxime and 1,2-cyclohexanedionedioxime as reagents. The presence of large amounts of aluminum and zinc must be avoided. Alkaline earths and magnesium do not interfere. All the iron is converted to the ferrous state by reduction with hydrazine. To determine the total iron add to 50 ml. of sample containing 1-4 mg. of iron, 1 gram of hydrazine sulfate, and 5 ml. of a saturated solution of dimethylglyoxime in 95 per cent ethanol. Heat to boiling. Add 10 ml. of concentrated ammonium hydroxide and continue to boil for 0.5 minute. Cool rapidly and dilute to 100 ml. Compare with a standard similarly prepared or measure the transmittance. To determine ferrous iron substitute 1 gram of tartaric acid for the hydrazine sulfate This maintains the ferric ion in solution in the presence of ammonium hydroxide and the ferric ion gives no color.

While iron can be determined by the brown color of ferrous sulfide in alkaline solution,[187] the method is of relatively minor importance. It is applied to water samples, and to various miscellaneous samples. Thus it determines 0.005-0.08 per cent of iron in aluminum and 0.005-0.1 per cent of iron oxide in pigments. One serious problem is that of getting the same particle size in the dispersion of sample and standard.

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[184] Georges Denigès, *Compt. rend.* 180, 519-20 (1925).

[185] L. Tschugaeff and B. Orelkin, *Z. anorg. Chem.* 89, 401-4 (1914); K. Nagaseko, *Mem. Coll. Sc., Kyoto Imp. Univ.* 11, 109,113 (1928); Paul Von Stein, *Chemist Analyst* 34, 15 (1945).

[186] Margaret Griffing and M. G. Mellon, *Anal. Chem* 19, 1017-20 (1947).

[187] L. W. Winkler, *Z. anal. Chem.* 41, 550 (1902); H. Ginsberg, *Metallwirtsch. Metallwiss. u. Metalltechn.* 16, 1107 12 (1937); K. Steinhauser and H. Ginsberg, *Z. anal Chem* 104, 385 90 (1936).

CHAPTER 18

NICKEL

Nickel is one of the rarer elements, although often associated in minerals with iron and copper. Contamination of foods from cooking utensils offers an occasional need of its estimation in organic materials, but samples are usually alloys or minerals. Very suitable methods of separation from other metals are available.

The methods of determination are relatively limited. The complexes with dimethylglyoxime, diethyldithiocarbamate, and ammonia are the main ones.

SAMPLES

Aluminum Alloys.[1] Treat 0.5 gram of alloy with 20 ml. of 10 per cent sodium hydroxide solution. Warm gently and, when reaction ceases, heat to boiling. Let cool somewhat and add 20 ml. of 1:1 nitric acid. Boil until solution is complete and the nitrous fumes are expelled. Dilute to a known volume and determine by the dimethylglyoxime method. The final solution contains the copper present in the original sample.

Nickel-chrome Alloys. A solution was prepared for determination of chromium (page 267) of which an aliquot may be used for nickel.

Aluminum-copper-nickel-manganese-iron Alloys. The sample solution was prepared for copper determination (page 80). Use the dimethylglyoxime method.

Steel and Cast Iron. The many kinds of steels and cast irons lead to a corresponding multiplicity of methods of preparation of samples. Dissolve[2] a 0.25-gram sample of ordinary steel in 10 ml. of 1:1 nitric acid and heat until oxides of nitrogen are expelled. For alloy steels

[1] Francesco Villani, *Ann. chem. applicata* **32**, 325-30 (1942); F. Sinigaglia *Allumino* **11**, 96-100 (1942); S. Bertoldi, *ibid.* **12**, 37-9 (1943).

[2] G. R. Makepeace and C. H. Craft, *Ind. Eng. Chem., Anal. Ed.* **16**, 375-8 (1944); cf. Gerd Maassen, *Die Chemie* **56**, 234-5 (1943); A. M. Dymov and O. A. Volodina, *Zavodskaya Lab.* **12**, 534-42 (1946).

dissolve with 20 ml. of an acid mixture containing 133 ml. of concentrated sulfuric acid and 167 ml. of 85 per cent orthophosphoric acid per liter. Then add 10 ml. of 1:1 nitric acid and heat until oxides of nitrogen are expelled. For stainless-type steels addition of up to 10 ml. of 1:1 hydrochloric acid will facilitate solution. Finally transfer to a volumetric flask of suitable size, according to nickel content, and dilute to volume for use of an aliquot.

Alternatively,[3] dissolve 0.5 gram of sample in 15 ml. of 1:2 nitric acid and boil gently until oxides of nitrogen have been removed. Add 10 ml. of 10 per cent ammonium persulfate solution and boil for 15 seconds. Add 1 ml. of methanol and 10 ml. of water. Add 25 ml. of concentrated ammonium hydroxide and boil for 1 minute. This precipitates chromium, molybdenum, aluminum, and vanadium with the iron. Filter and use as sample for the dimethylglyoxime method.

As another method of oxidation,[4] to a 0.5-gram sample add 15 ml. of 1:3 nitric acid and warm. When solution is complete add 1 per cent potassium permanganate solution dropwise until a faint pink persists during boiling for 1 minute. Add a 20 per cent solution of ferrous ammonium sulfate until the precipitate of manganese dioxide is just redissolved. Boil until oxides of nitrogen are removed and let cool. Transfer to a 100-ml. volumetric flask and dilute to volume. Use an aliquot by the dimethylglyoxime method.

A variation[5] of this is to heat 0.2 gram with 15 ml. of 1:2 nitric acid until the initial vigorous reaction is over. Add 10 ml. of 72 per cent perchloric acid and heat slowly until solution is complete and perchloric acid is refluxing freely on the wall of the beaker. With cast-iron samples it may be necessary to add a second portion of nitric acid in order to complete solution and oxidation of carbides. Cool and dilute with water. Either use the entire solution or dilute to a known volume and take an aliquot containing 0.02-0.08 mg. of nickel. Separate the nickel by dimethylglyoxime extraction (page 343) and determine by diethyldithiocarbamate

Manganese Steel.[6] Treat a 1-gram sample with 30 ml. of concentrated nitric acid and evaporate nearly to dryness. Add 20 ml. of the

[3] C. G. Hummon, *Steel* 114, *No.* 25, 97 (1944); cf. H. L. Mawzy and H. Yellin, *Metal Progress* 45, 689-90 (1944).

[4] O. A. Yakovleva, *Zavodskaya Lab.* 11, 47-52 (1945).

[5] O. R. Alexander, Edith M. Godar, and N. J. Linde, *Ind. Eng. Chem., Anal. Ed.* 18, 206-8 (1946).

[6] B. Jones, *Analyst* 54, 582-9 (1929).

concentrated acid and 5 ml. of 20 per cent chloric acid. Boil for 5 minutes and again add the same amounts of reagents. Boil for 5 minutes, let cool, and filter through an inorganic filter. Wash the residue of manganese dioxide on the filter with 1:1 nitric acid. Evaporate the filtrate and washings nearly to dryness, take up with water, add 10 ml. of concentrated hydrochloric acid, and dilute to a known volume. Use an aliquot for determination by the dimethylgloxime method.

Copper and Copper Alloys.[7] Dissolve a 1-gram sample in the appropriate solvent. If the sample contains less than 0.1 per cent of lead, heat with 25 ml. of a mixture containing 50 ml. of concentrated sulfuric acid in 125 ml. of water, to which 35 ml. of concentrated nitric acid has been added. If more than 0.05 per cent of tin is present add to the sample 15 ml. of a mixture of 20 ml. of 48 per cent hydrofluoric acid solution and 180 ml. of saturated boric acid solution. Add 30 ml. of 1:1 nitric acid. In any case let the reaction proceed in the cold as far as possible, then warm to about 90° to complete and expel colored fumes.

For samples high in tin add 15 ml. of a mixture of 10 ml. of bromine per 100 ml. of concentrated hydrobromic acid to the solid sample. Avoid loss of free bromine until solution is complete. Add 10 ml. of 72 per cent perchloric acid and heat on a hot plate to expel arsenic, antimony, and tin as bromides. Finish over a free flame, swirling constantly to avoid bumping. Evaporate slowly to copious white fumes to expel all the hydrobromic acid. Finally evaporate to 5 ml., let cool, and add 10 ml. of water. Heat to boiling to dissolve soluble salts but ignore cloudiness of the solution. Add 2 ml. of concentrated nitric acid.

After solution by one of these methods, boil until brown fumes are no longer given off. Dilute to about 175 ml. and insert electrodes. Pass a current of 0.5 ampere per sq. dm. overnight or 4 amperes per sq. dm. for gauze electrodes for 2.5 hours. When the solution is colorless, wash down the electrodes, cover glass, and sides of the beaker to raise the level and continue to see whether copper deposits on the newly covered surface. Continue until the copper removal is complete. Remove the electrodes quickly while washing them down with water. If residual lead sulfate, manganese dioxide, etc., are present, filter the solution. Dilute to 200 ml. and use aliquots for determination by the dimethylglyoxime method.

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[7] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 332-4. American Society for Testing Materials, Philadelphia, Pa. (1946).

Alternatively, prepare the sample as described for determination of iron (page 282).

Bronzes.[8] Add 2 ml. of 1:1 nitric acid to 0.1 gram of fine drillings and heat until solution is complete. Evaporate until a greenish color is evident and dilute with 20 ml. of distilled water. Add 5 ml. of 5 per cent ammonium hypophosphite solution, and boil for 3 minutes. This precipitates the major part of the copper, although not quantitatively, and thus avoids later interference by that element. Let cool, filter into a 250-ml. volumetric flask, and wash with tepid water until the volume is about 150 ml. Cool and dilute to volume. Use a suitable aliquot as sample for the dimethylglyoxime method. Usually 25 ml. is suitable for less than 5 per cent of nickel.

Slag.[9] Heat 1 gram of slag with 30 ml. of concentrated hydrochloric acid and 10 ml. of concentrated nitric acid. When reaction has ceased add 3 ml. of 48 per cent hydrofluoric acid and boil until solution is complete. Add 50 ml. of saturated bromine water and then an aqueous suspension of zinc oxide until precipitation of iron is complete. Boil for 5 minutes to insure complete precipitation, let cool, and dilute to 250 ml. in a volumetric flask. Filter into a dry flask and use an aliquot as sample. In development of color by the dimethylglyoxime method it will be necessary first to acidify with 1:1 hydrochloric acid and later add sufficient ammonium hydroxide to dissolve the zinc hydroxide as the ammonia complex.

Iron Ore.[10] Dissolve as much as possible of a 1-gram sample by heating with 15 ml. of concentrated hydrochloric acid and 5 ml. of concentrated nitric acid. When reaction is complete, dilute to about 100 ml. and filter to remove insoluble matter such as silica. Wash the precipitate thoroughly and discard. Add 2 grams of ammonium chloride to the combined filtrate and washings. Heat this to boiling and add 1:1 ammonium hydroxide until a flocculent precipitate of ferric hydroxide is obtained. This occludes some nickel. Filter and without washing set the filtrate aside. Dissolve the ferric hydroxide in a minimum volume of hot 1:10 hydrochloric acid. Precipitate the iron as before, filter, and save the filtrate. Dissolve and reprecipitate once more. Discard the precipitate. Combine the filtrates and concentrate to under 250 ml.

[8] G. Haim and B. Tarrant, *Ind. Eng. Chem., Anal. Ed.* 18, 51-2 (1946).
[9] P. F. Federov and S. M. Yanovskii, *Zavodskaya Lab.* 7, 478-9 (1938).
[10] E. N. Deichman, *J. Applied Chem.* (U.S.S.R.) 8, 1096-9 (1935).

Dilute to volume in a volumetric flask and use suitable aliquots. The same solution is suitable for determination of cobalt.

Silicates.[11] To a 0.25-0.5 gram sample in platinum add 2 ml. of water, 0.5 ml. of 72 per cent perchloric acid, and 2.5-5.0 ml. of 48 per cent hydrofluoric acid. Evaporate to dryness. Take up the residue in 2 ml. of water and 0.5 ml. of 72 per cent perchloric acid, and again evaporate to dryness. Add 6 ml. of 1:5 hydrochloric acid and heat until all soluble material is dissolved. Add 5 ml. of 10 per cent sodium citrate solution and neutralize to litmus with concentrated ammonium hydroxide. Add 3 drops excess and filter to remove the insoluble residue of the mineral. Set aside the filtrate as a first sample solution. Wash the paper, dry, and ignite. Fuse the ash with 0.1 gram of sodium carbonate, take up in water, and add 1:10 hydrochloric acid to facilitate solution. Add 2 ml. of 10 per cent sodium citrate solution and set this aside as a second sample solution.

Add 2 ml. of a 1 per cent solution of dimethylglyoxime in 95 per cent ethanol to the first sample solution and extract it with 3 ml. of chloroform. Separate the extract and repeat twice more. Similarly treat the second sample solution if it shows any nickel present. Combine all the chloroform extracts and shake vigorously with 10 ml. of 1:50 ammonium hydroxide. Separate the chloroform layer and wash the alkaline layer with 1 ml. of chloroform, adding this to the balance of the chloroform extract. Now the nickel has been extracted from other metals and is present in the chloroform layer as the dimethylglyoxime. Discard the aqueous layers.

Shake this chloroform extract with 4 ml. of 1:25 hydrochloric acid to extract the nickel as chloride. Repeat this extraction twice with 2-ml. portions of the acid. Extract further, if necessary, unless all the nickel has been removed. Dilute the acid extracts to a known volume, which may be as small as 10 ml., and use aliquots.

Foods and Biological Materials.[12] Dry a sample containing 0.005-0.1 mg. of nickel on a steam bath or hot plate. Add 25 ml. of concentrated nitric acid and heat gently to initiate oxidation. When the first rapid action is over, let cool, add 5 ml. of concentrated sulfuric acid, and heat again. As the mixture darkens and charring begins, add 5 ml.

[11] E. B. Sandell and R. W. Perlich, *Ind. Eng. Chem., Anal. Ed.* 11, 309-11 (1939); cf. L. A. Gulyaeva, *J. Applied Chem.* (U.S.S.R.) 18, 726-7 (1945).

[12] O. R. Alexander, Edith M. Godar and N. J. Linde, *Ind. Eng. Chem., Anal. Ed.* 18, 206-8 (1946).

more of concentrated nitric acid from time to time. When all organic matter appears to be destroyed, heat to fumes of sulfur trioxide. Let cool and add 1 ml. of 72 per cent perchloric acid. Heat again to copious fumes of sulfur trioxide. Let cool and take up with about 25 ml. of water.

Separate the nickel by dimethylglyoxime extraction and determine by diethyldithiocarbamate.

Concentration of Nickel. Various methods of separation of nickel are available in addition to those described specifically for particular kinds of samples.

As the Ferrocyanide.[13] Adjust the solution until just faintly acid with hydrochloric acid. Add 1 ml. of a 1 per cent solution of zinc chloride or zinc sulfate as collector. Add 2 grams of ammonium chloride. By addition of potassium ferrocyanide the insoluble ferrocyanides of nickel and zinc are precipitated. Filter and wash free of iron. Ignite to oxides. Dissolve the nickel by heating with 5 ml. of hot 1:1 hydrochloric acid to dissolve the nickel oxide with some other metals. Dilute to a known volume for use of an aliquot.

Extraction as Nickel Dimethylglyoxime. If the sample has not already been oxidized, adjust to faint acidity and add saturated bromine water to a faint yellow and 2 ml. in excess. Prepare an ammonium citrate buffer by dissolving 200 grams of diammonium citrate in about 600 ml. of water. Add 1:1 ammonium hydroxide until the pH is 9.0-9.5. In a liter separatory funnel add 10 ml. of dimethylglyoxime solution prepared by dissolving 0.25 gram of dimethylglyoxime in 50 ml. of 95 per cent ethanol and diluting to 250 ml. with water. Extract with three 30-ml. portions of chloroform and discard the extracts. Dilute the buffer to 1 liter

Add 10 ml. of the buffer to the sample containing 0.02-0.08 mg. of nickel and dilute to about 40 ml. Add 1:1 ammonium hydroxide until the pH is about 8.5-9.0 as shown by reaction with phenol red. Add 5 ml. of the dimethylglyoxime reagent and 10 ml. of chloroform. Shake vigorously for 1 minute. Withdraw all but a few drops of the solvent into a second separatory funnel containing 25 ml. of 1:50 ammonium hydroxide. Extract the sample with a further 5-ml. portion of chloroform and withdraw as completely as possible. Discard the aqueous sample.

[13] Erich Reichel and Ludwig Stuzin, *Z. anal. Chem.* 113, 389-419 (1938).

Nickel has been separated from iron, aluminum, cobalt, etc., but some copper may have been extracted.

Shake the combined chloroform extracts with the dilute ammonium hydroxide for 1 minute, let separate, and draw off the aqueous layer to waste. Wash down the sides of the funnel with water, withdraw this, and discard. This alkaline wash will have removed any copper which was coextracted.

Add 25 ml. of 1:25 hydrochloric acid to the chloroform layer and shake thoroughly. Withdraw the acid layer containing the nickel and discard the chloroform layer. Shake the acid layer with 5 ml. of carbon tetrachloride and discard this extract. Be sure that separation is complete. The acid extract is now ready for use as sample for the diethyldithiocarbamate method.

Precipitation as Nickel Dimethylglyoxime.[14] Remove copper and lead electrolytically as described (page 340). To the solution add 10 ml. of 25 per cent tartaric acid solution. Neutralize with concentrated ammonium hydroxide and add 1 ml. excess. Heat to about 70°, and add 0.4 ml. of a 1 per cent solution of dimethylglyoxime in ethanol for each mg. of nickel expected plus 3-5 ml. in excess. Add the reagent to the solution with stirring; do not run it down the wall of the beaker. Let cool to room temperature with occasional stirring or let it cool overnight.

Filter the glyoxime so obtained on a Gooch crucible. When well washed with cold water, dissolve from the filter with concentrated nitric acid and evaporate to a few drops in a casserole. Add 2-3 ml. of concentrated nitric acid and again evaporate to insure destruction of organic matter. Take up with water and either use as sample or dilute to a known volume to obtain suitable aliquots.

Removal of Iron as Fluoride. Follow the technic described for copper (page 107).

Standard

As standard, dissolve 0.4479 gram of nickel sulfate, $NiSO_4 \cdot 6H_2O$, 0.6730 gram of nickel ammonium sulfate, $NiSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, or an equivalent amount of other nickel salt in water, and dilute to 1 liter. This contains 0.1 mg. of nickel per ml. and may be further diluted to a basis of 0.01 mg. per ml. if necessary.

[14] Gilbert H. Ayres and Francene Smith, *Ind. Eng. Chem., Anal. Ed.* 11, 365-7 (1939).

Nickel by Dimethylglyoxime

In an alkaline medium, nickel and dimethylglyoxime, diacetyldioxime, in the presence of an oxidizing agent form a wine-red complex suitable for colorimetric estimation.[15] Nickel is present in this at a valence higher than two since it will liberate iodine from potassium iodide in acid solution and will not precipitate as sulfide. It is believed to be nickelic dimethylglyoxime. The reaction is comparable in sensitivity to formation of the ferric thiocyanate complex.

The original method used lead dioxide as oxidizing agent. Sodium hypochlorite has also been applied but bromine water, iodine, or perchloric acid are most common and convenient. Excess oxidizing agent in alkaline solution is essential to high sensitivity. Ammonia is used to raise the pH to the necessary level. Increase of the amount of dimethylglyoxime or ammonia increases the stability of the color.[16] The reaction is empirical and repetition depends on adherence to a carefully defined procedure. In an aqueous solution development of color is slow and unreliable. By use of 50 per cent ethanol, development of color is prompt and no fading occurs in the first 30 minutes.

The following ions precipitate with the reagent and must be removed or tied up by complex formation: chlorostannous, chlorostannic, iodide, permanganate, silicate, thiosulfate, vanadate, aluminum, antimony, barium, beryllium, bismuth, cerium, chromium, copper, ferrous, ferric, lead, magnesium, manganese, mercurous, mercuric, platinum, silver, strontium, thorium, titanium, uranyl, zirconium. The color of auric, cobaltous, and dichromate ions interferes.

Ferric ion is often tied up in moderate amounts as an organic complex. For this tartaric acid is preferable to citric but must be of the highest purity; some impurities inhibit color development with nickel. Purity of the dimethylglyoxime is also essential. When iron is precipitated as hydroxide, by forming the dimethylglyoxime complex before precipitation by ammonia, loss of nickel by sorption is minimized.[17] An alternative to precipitation of ferric ion in the presence of preformed

[15] Fritz Feigl, *Ber.* 57B, 759 61 (1924); R. Juza and R. Langheim, *Angew. Chem.* 50, 255 9 (1937); Kurt Dietrich and Karl Schmitt, *Z. anal. Chem.* 109, 25-31 (1937); W. M. Murray, Jr, and S. E. Q. Ashley, *Ind. Eng. Chem*, *Anal. Ed.* 10, 1 5 (1938); I. M. Korenman and G. D. Voronov, *Zavodskaya Lab.* 8, 664 5 (1939); G. R. Makepeace and C H. Craft, *Ind Eng Chem., Anal. Ed.* 16, 375-8 (1944); Emanuel Passamaneck, *ibid.* 17, 257 8 (1945); A. M. Mitchell and M. G. Mellon, *ibid.* 17, 380-2 (1945); John H. High, Analyst 70, 258 9 (1945).

[16] G. V. L. N. Murty and N. B. Sen, *Proc. Indian Acad. Sci.* 21A, 73-5 (1945).

[17] K. Dietrich and Karl Schmitt, *Z. anal. Chem.* 109, 25-31 (1937).

nickel dimethylglyoxime is precipitation of the iron with an alkaline dimethylglyoxime reagent.[18]

Small amounts of nickel are separated from large amounts of cobalt by precipitation as the dimethylglyoxime and the separated precipitate is dissolved in a small volume of 1:4 hydrochloric acid.[19] For study of transmittance it is desirable to have practically none below 450 mμ. There is some absorption by iron, even in citrate buffer, above 500 mμ. The optimum is at 530 mμ, at which the system follows Beer's law. Good agreement is obtained with gravimetric results.[20]

The nickel compound is soluble in some organic solvents which gives a method of concentration of small amounts. Thus this procedure is applicable to nickel in chrome plating baths.[21] By extraction with chloroform the copper and nickel compounds are separated[22] and then the copper compound can be washed out of the chloroform layer with ammonia (page 343). An alternative is to precipitate copper as the sulfide before extraction of the nickel. Precipitation of the nickel dimethylglyoxime on barium sulfate has been proposed as a method of reading the color.[23]

Procedure. *Direct Determination.* Adjust an aliquot of sample containing 0.5 mg. of nickel or less to faint acidity by addition of 1:1 hydrochloric acid or 1:1 ammonium hydroxide. If the sample is of steel or iron, or contains substantial amounts of ferrous or ferric ions, add 5 ml. of a 20 per cent tartaric acid solution. Add saturated bromine water until a faint yellow persists and 2 ml. in excess. Add 10 ml. of concentrated ammonium hydroxide and mix well.

If no precipitation occurs, transfer to a 100-ml. volumetric flask. If precipitation occurs filter into the flask. In the latter case redissolve the precipitate from the paper with the minimum amount of 1:1 hydrochloric acid, add 1 ml. of bromine water, and reprecipitate with 4 ml. of concentrated ammonium hydroxide. Filter this and wash with water, receiving the filtrate and washings in the 100-ml. volumetric flask containing the previous filtrate.

Add 35 ml. of 95 per cent ethanol and 20 ml. of a 0.1 per cent solution

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[18] O. A. Yakovleva, *Zavodskaya Lab.* **11**, 471-2 (1945).

[19] V. M. Peshkova, *ibid.* **8**, 921-5 (1939); cf. D. F. Phillips and L. L. Edwards, *Metal Ind.* (London) **66**, 409-10 (1945).

[20] K. V. Troitskaya, *Uchenye Zapiski Kazan. Gosudarst. Univ.* **97**, 67-81 (1937)

[21] D. Gardner Foulke, *Monthly Rev. Am. Electroplaters' Soc.* **32**, 7-10 (1945).

[22] A. J. Hall and R. S. Young, *Analyst* **71**, 479-82 (1946).

[23] Pierre Süe, *Ann. chim. anal.* **28**, 26 (1946).

of dimethylglyoxime in ethanol. Dilute to volume with water, mix well, and read the transmittance at 530 mμ within 15 minutes; or balance against a standard prepared at the same time.

Alternatively with a solution of a steel sample, transfer an aliquot representing about 0.1 gram of sample to a 100-ml. volumetric flask. Add 10 ml. of saturated bromine water. Next add 20 ml. of a 1 per cent solution of dimethylglyoxime in 5 per cent sodium hydroxide solution. Dilute to volume and mix. Filter, discarding the first part of the filtrate because of possible sorption on the paper, and read the transmittance at 475 mμ, or compare with standards.

By Extraction.[24] Transfer a sample containing 0.002-0.03 mg. Make it substantially neutral with 1:1 ammonium hydroxide for 1:1 hydrochloric acid and evaporate to dryness. Take up in 2 ml. of water and add 1 ml. of 50 per cent potassium citrate solution. Add 5 drops of concentrated ammonium hydroxide and follow with 1.6 ml. of a 1 per cent solution of dimethylglyoxime in ethanol. Mix well and let stand overnight for complete precipitation. Then filter on an inorganic filter, wash well with hot water, and dry for 1-2 hours at 100° Dissolve the precipitate from the filter by suction, using pyridine as solvent and a graduated tube as receiver. Dilute to 3-10 ml. according to nickel content and subsequent procedure to be used. Compare with a standard by balancing, or read the transmittance photometrically. The maximum is in the region of 360 mμ.

Nickel by Diethyldithiocarbamate

The yellow-green complex which nickel forms with diethyldithiocarbamate is extractable with amyl alcohol and the extract so obtained is suitable for photometric estimation of nickel.[25] Since nickel, copper, bismuth, cobalt, and iron form colored compounds with diethyldithiocarbamate which are soluble in organic solvents, nickel must be isolated, preferably by extraction as the dimethylglyoxime complex. In ammoniacal citrate solution the reaction with iron is prevented. Extraction under those conditions gives the nickel, possibly accompanied by a little copper. An intermediate washing of the extract with dilute ammonium hydroxide serves to remove all the copper.

The visual sensitivity is low as the maximum absorption is on the edge of the visual spectrum. The system conforms to Beer's law and

[24] Emanuel Passamaneck, *Ind. Eng. Chem., Anal. Ed.* **17**, 257-8 (1945).

[25] O. R. Alexander, Edith M. Godar, and N. J. Linde, *ibid.* **18**, 206-8 (1946).

the method will determine 0.001 mg. or more of nickel with satisfactory accuracy.

Procedure. The nickel should have been isolated by the dimethylglyoxime method (page 343). Prepare an ammonium citrate buffer by dissolving 200 grams of diammonium citrate in about 600 ml. of water. Add 1:1 ammonium hydroxide until the pH is 9.0-9.5. Transfer to a liter separatory funnel and add 10 ml. of a filtered 0.2 per cent solution of diethyldithiocarbamate in water. Extract with successive 20-ml. portions of chloroform until an extract is colorless. Add 5 ml. more of the carbamate reagent and again extract as before. If the solvent layer remains yellow add more reagent and again extract. Finally dilute to 1 liter.

Add 5 ml. of this buffer to the sample, and 1:1 ammonium hydroxide until alkaline to litmus and about 1 ml. excess. Add 10 ml. of isoamyl alcohol and 5 ml. of filtered 0.2 per cent aqueous solution of diethyldithiocarbamate. Shake for 2 minutes and withdraw the aqueous layer to waste. Read the transmittance of the solvent layer at 385 mμ. When standards are prepared, dilute to volume with 1:25 hydrochloric acid before carrying through the procedure.

Nickel as the Ammonia Complex

The blue to violet complex formed by nickelous ion and ammonia is suitable for colorimetric estimation.[26] The system conforms to Beer's law and gives the maximum absorption around 582 mμ. Samples in which nickel has been precipitated as the dimethylglyoxime, separated and decomposed by nitric acid, are suitable. The blue color is given around 1:10 ammonium hydroxide, changing appreciably to violet at around 1:6. Ammonium salts up to 5 per cent do not affect the color. The interference by cobalt cannot be overcome by filters and it must therefore be substantially absent. The method is applicable at 5-4000 ppm. and sensitive to 1 per cent in the range of 500-1500 ppm. The color is not sufficient to permit visual matching with any high degree of accuracy.

No fading occurs in a year. Copper interferes by forming a colored complex ion. Such ions as chromate affect the color by their own hue.

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[26] R. Fieber, *Chem Ztg.* 24, 393 (1900); *J. Soc. Chem. Ind.* 19, 563 (1900); Gilbert H. Ayres and Francene Smith, *Ind. Eng. Chem., Anal Ed.* 11, 365-7 (1939); J. P. Mehlig, *ibid.* 14, 289-92 (1942); J. P. Mehlig and R. E. Kitson, *ibid.* 15, 606 (1943).

Some such as cyanide, citrate, pyrophosphate, and salicylate form complexes which change the hue. Ions which precipitate as insoluble hydroxides must be removed. Unlike the copper system, ammonium chloride does not intensify the color. The method is applicable to nickel baths suitably diluted.[27]

Procedure. Transfer an aliquot of sample containing 0.05-0.15 mg. of nickel. If substantial amounts of cobalt are present nickel should have been separated by a dimethylglyoxime technic (page 343). Transfer to a 100-ml. volumetric flask and approximately neutralize with 1:1 ammonium hydroxide or 1:1 hydrochloric acid. Add 10 ml. of concentrated ammonium hydroxide and dilute to volume. If precipitation occurs filter or centrifuge to obtain the clear solution. Compare with a standard similarly prepared or read the transmittance at around 580 mμ.

Miscellaneous

In the absence of other metals giving colored sulfides the general method (page 40) is applicable to nickel. A general method of separation of nickel from interfering ions is by precipitation as the dimethylglyoxime, filtration, washing, ignition, and solution in acid.[28] With sodium sulfide as the precipitating agent a saturated solution of basic nickel hydrosulfide contains 0.8×10^{-5} per cent of nickel and, with excess hydrogen sulfide present, has the formula $Ni(OH)SH$.[29]

When nickel in ammoniacal solution is acted on by potassium thiocarbonate a rose red to dark red or brown color is produced, suitable for colorimetric estimation.[30] Copper and cadmium interfere but may be precipitated by hydrogen sulfide. Manganese and iron must be removed, cobalt must be oxidized. Zinc forms a white precipitate with the reagent. The sample should contain less than 2.5 mg. of nickel, as chloride, sulfate, or nitrate in acid or neutral solution. In 20 ml., 0.17 mg. of nickel will give a dark red, and 0.0008 mg. a light yellow. The reagent alone gives a yellowish color. The most accurate results are obtained from an aliquot containing 0.02-0.1 mg. Oxidize a suitable portion of sample solution with excess bromine-water, neutralize, and add concentrated ammonium hydroxide in slight excess. This precipitates manganese, iron, and aluminum. Boil, cool, make up to a standard volume and filter

[27] Hermann Wagner, *Z. metall u. Schmuckw.-Fabrik Verchrom.* 21, 342 (1940).
[28] Erich Reichel and Ludwig Stuzin, *Z. anal. Chem.* 113, 389-410 (1938).
[29] Alexander Mickwitz, *Z. anorg. allgem. Chem.* 196, 113-19 (1931).
[30] V. Lindt, *Z. anal. Chem.* 53, 165-75 (1914).

through a dry filter into a dry receiver. To 20 ml. of this filtrate, or a lesser amount diluted to 20 ml. with 1:50 ammonium hydroxide, add 0.5 ml. of 4 per cent aqueous potassium thiocarbonate solution and mix. The color develops at once. The permanence of the color is questionable over a period of time. Above 0.17 mg. per ml. a precipitate forms in about 4 hours.

Potassium dithiooxalate gives a magenta color with nickel in dilute solution.[31] The system conforms to Beer's law. Manganese affects the color when present in relatively large amounts. Iron must be removed, as it gives a deep purple. Cobalt must be absent. Other ions which give color are antimony, bismuth, cadmium, cobalt, copper, mercury, silver, tin, palladium, platinum, zinc, cerium, gold, thallium, titanium, and vanadium. The color develops in neutral solution, or up to 0.1 molar acidity. If the nickel is less than 1 ppm., an acidity over 0.01 molar gives a yellowish brown instead of pink. An average error is about 2 per cent. Both the reagent and the complex formed are unstable. To develop the color, transfer an aliquot to a 50-ml. comparison cylinder, dilute nearly to volume and add 1 ml. of 0.1 per cent aqueous potassium dithiooxalate solution. Compare with a standard similarly prepared.

The reaction of nickel with a formaldoxime reagent to give a brownish tint may be used for its estimation in cobalt salts.[32] The method will detect 0.1 per cent of nickel in a salt. As reagent mix 7 grams of hydrazine hydrochloride, 15 ml. of water, and 3 grams of trioxymethylene. Heat slowly to boiling, boil until clear, and cool. To 20 ml. of a solution of the cobalt salt containing 50 mg. of cobalt per liter, and a similar standard of known nickel-free salt, add 2 drops of the reagent and mix. Add 4 drops of 40 per cent sodium hydroxide solution and mix. The nickel-free solution will be pale yellow. If the sample contains nickel it will be brown. Compare with a series of standards prepared from cobalt salt solution to which known amounts of nickel were added.

The nickel derivative of 8-hydroxyquinoline in chloroform can be read directly at 395 mμ.[33] The reagent gives a measurable absorption. Extraction begins above pH 2.5 but is complete only above 6.7. Deviations from Beer's law are minor up to 10 ppm.

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[31] H. O. Jones and H. S. Tasker, *J. Chem. Soc.* 95, 1904-9 (1909); L. T. Fairhall, *J. Ind. Hyg.* 8, 528-33 (1926); John H. Yoe and Floyd H. Wirsing, *J. Am. Chem. Soc.* 54, 1866-76 (1932).

[32] Georges Denigès, *Bull. soc. pharm. Bordeaux* 70, 101-7 (1932).

[33] Therald Moeller, *Ind. Eng. Chem., Anal. Ed.* 15, 346-9 (1943).

Reading the transmittance of solutions of nickel salts with a red filter gives a method of measurement of concentration comparable in accuracy with electrolytic determination.[34] Interference by copper is avoided by addition of sulfuric acid and thiourea. Orthophosphoric acid will prevent interference by iron. A range of 0.050-0.50 grams of nickel in the cell, of variable size, is applicable.

[34] J. Kinnunen, *Metall. u. Erz* **41**, 158 (1944).

CHAPTER 19

COBALT

Cobalt is, in occurrence, associated with iron, copper, and nickel. The major samples are alloys and minerals. Biological occurrence has to be considered, and its presence as a dryer in protective coatings is of importance. Separation from nickel and iron is well developed.

A blue color with thiocyanate and colloidal dispersions with nitrosonaphthols are the classical methods of determination. Recent methods with more complex organic reagents are important so that currently there is no one outstanding method.

Samples

Steel.[1] Dissolve 0.5 gram of steel in the form of fine drillings, turnings, or millings by heating with a mixture of 10 ml. of concentrated orthophosphoric acid and 10 ml. of concentrated sulfuric acid. Cool, add 5 ml. of 1:1 nitric acid, and evaporate to fumes of sulfur trioxide. Cool, dilute to dissolve any salts, and make up to 100 ml. Take an aliquot for determination with nitroso-R salt as the reagent.

If the sample must have ferric and similar ions removed, dissolve 2 grams of steel in 50 ml. of 1:1 hydrochloric acid. To the solution add 1:1 nitric acid, dropwise, to oxidize iron, and boil until the solution is yellow in color. Evaporate to half the volume, add 50 ml. of 1:1 hydrochloric acid, and again evaporate to 25 ml. Cool and add 100 ml. of water. Transfer to a 500-ml. volumetric flask and add freshly prepared zinc oxide suspension in slight excess to precipitate iron, aluminum, manganese, nickel, etc. Dilute to volume and filter. Use suitable aliquots, usually 25 ml.

Stainless Steel.[2] Treat a 0.25-gram sample with 5 ml. of concentrated hydrochloric acid, 5 ml. of concentrated nitric acid, and 0.1 ml. of 40 per cent hydrofluoric acid. After the sample dissolves with gentle heating, boil for several minutes. Let cool somewhat and add 10 ml. of 72 per cent perchloric acid. Heat until fumes of perchloric acid are

[1] F. W. Haywood and A. A. R. Wood, *J. Soc. Chem. Ind.* 62, 37-9 (1943).
[2] Harry M. Putsché and W. Francis Malooly, *Anal. Chem.* 19, 236-8 (1947).

given off. The chromium will be present as chromic acid. Cool and add 25 ml. of water. Add 1:1 ammonium hydroxide nearly to neutrality and cool. Transfer to a 250-ml. volumetric flask and add zinc oxide suspension in water until precipitation of iron is complete. Dilute to volume and let settle. Pipet 25 ml. of the clear upper layer, add 5 ml. of saturated sulfurous acid, and boil off excess sulfur dioxide. Use this as a sample for development of color with thiocyanate.

Alloys. *Copper and Iron Absent.* Dissolve 0.2 gram of alloy in 5 ml. of concentrated nitric acid. Evaporate to dryness on a water bath and take up with water. Dilute to a known volume and use an aliquot.

Copper and Iron Present. Dissolve 0.2 gram of alloy in 5 ml. of concentrated nitric acid and evaporate to dryness on the water bath. Take up with 5 ml. of 1:4 hydrochloric acid and dilute to 50 ml. with water.

Precipitate copper by hydrogen sulfide in hot solution. Filter, boil off hydrogen sulfide, and oxidize the filtrate with 1 ml. of concentrated nitric acid. Evaporate to dryness on the water bath. Take up the residue with a few drops of 1:40 hydrochloric acid. Neutralize the clear solution with 1 per cent sodium carbonate solution and add 1 ml. of 10 per cent sodium acetate solution while hot. Boil for 10 minutes. Let the precipitate of basic ferric acetate settle, filter, and wash with hot water. Add 5 ml. of concentrated hydrochloric acid to the filtrate, and evaporate to dryness. Dissolve the residue in water, dilute to a known volume and use on aliquot.

Blister Copper and Refined Copper. As sample use the solution from electrolytic deposition. Evaporate a suitable amount, according to probable cobalt content, to fumes of sulfur trioxide. Complete as for copper ores starting at "Cool, dilute to about 25 ml., and boil . . ." (page 355).

Alternatively prepare as for nickel determination (page 340) until ready for colorimetric estimation of nickel. Make the concentrated filtrates alkaline with 1:1 ammonium hydroxide and add 1 ml. of an aqueous 1 per cent dispersion of dimethylglyoxime. Let stand to precipitate, filter, wash, and discard the precipitate. Make the combined filtrate and washings definitely acid with 1:1 hydrochloric acid and heat to boiling. Add 70 per cent perchloric acid dropwise until the excesss of dimethylglyoxime has been destroyed. Neutralize the solution with 1:1 ammonium hydroxide, dilute to a known volume, and use aliquots,

Iron Ore. Prepare for determination of nickel (page 341) and estimate cobalt in another aliquot.

Cobalt Ores or Concentrates.[3] For samples containing 0.16-3.2 per cent of cobalt use 0.5 gram, for lower percentages use 2 grams. Add 10 ml. of concentrated nitric acid and 20 ml. of concentrated hydrochloric acid, and if necessary a few drops of bromine or hydrofluoric acid to promote solution. If there is a high iron content add a few crystals of potassium chlorate. Evaporate to dryness but do not bake. Take up in approximately 25 ml. of water and dilute to such a volume that the cobalt will be 0.01-0.1 mg. per ml. In dilution add 1 ml. of concentrated hydrochloric acid for each 50 ml. of final dilution. This provides a pH of 0.9-1.0 suitable for estimation as the thiocyanate.

Cobalt Oxide in the Presence of Cobalt Sulfide.[4] The determination consists primarily in a method of separation from mill feed or tailings. To a 0.25-gram sample add 6 ml. of 1:9 hydrochloric acid saturated with sulfur dioxide. After the initial effervescence has ceased, add 0.25 ml. of 48 per cent hydrofluoric acid. Shake every 10 minutes for a half minute during the first hour. Let stand for an hour, and again shake every ten minutes during the third hour. Filter with the aid of paper pulp and wash with hot water. Add 5 ml. of 1:1 sulfuric acid and evaporate to sulfur trioxide fumes. Cool, take up in 25 ml. of water, and boil to insure complete solution. Pass in hydrogen sulfide to precipitate copper sulfide, and filter. Wash the filter with 1:20 sulfuric acid. Boil off hydrogen sulfide from the combined filtrate and washings. Dilute to a known volume and use an aliquot as sample. For the nitroso-R-salt method evaporate to sulfur trioxide fumes before using as sample.

Iron-nickel Ores.[5] Heat a sample of 0.5-2 grams with 8-15 ml. of concentrated hydrochloric acid and an equal volume of concentrated nitric acid Evaporate the acid and take up the residue in 10-20 ml. of 1:10 hydrochloric acid. Without filtering off any silicious residue, add 1:1 ammonium hydroxide to a slight permanent turbidity. Add concentrated hydrochloric acid dropwise until the ferric hydroxide redissolves. The sample so prepared is designed for determination by the thiocya-

[3] R. S. Young and A. J. Hall, *Ind. Eng. Chem., Anal. Ed.* 18, 264-6 (1946); *cf.* V. M. Zvenigorodskaya, *Zavodskaya Lab.* 11, 1022-7 (1945).

[4] R. S. Young, A. J. Hall and H. L. Talbot, *Am. Inst. Mining Met. Engrs., Metal Technol.* 13, Tech. Pub. No. 2050, 5 pp. (1946).

[5] V. M. Zvenigorodskaya, *Zavodskaya Lab.* 7, 1350-5 (1938).

nate method. The residue need not be filtered out for that method. The presence of both iron and nickel renders it unsuitable for many methods.

Copper Ores.[6] For mill feed and tailings use a 0.25-gram sample. For oxide ore and copper concentrate use a 0.5-gram sample and later take a one-tenth aliquot. For copper reverberatory slag and matté use a 0.5-gram sample but a one-twentieth aliquot.

Treat the sample with 10 ml. of concentrated nitric acid and 20 ml. of concentrated hydrochloric acid, adding a few drops of bromine to expedite reaction. When silica is present the addition of hydrofluoric acid is desirable. After decomposition is complete, add 5 ml. of 1:1 sulfuric acid and evaporate to copious fumes of sulfur trioxide. Add no more reagents which will not be volatilized than essential. This will avoid correspondingly objectionable amounts of inorganic salts later.

To the cooled residue add about 20 ml. of 1:10 hydrochloric acid and boil until soluble salts are dissolved. Dilute to 30 ml. and pass in hydrogen sulfide for 10 minutes. Filter and wash the residue on the paper with 1:50 hydrochloric acid saturated with hydrogen sulfide. Boil the filtrate and washings to expel hydrogen sulfide. Add 5 ml. of concentrated nitric acid and evaporate to fumes of sulfur trioxide.

Cool, dilute to about 25 ml., and boil to dissolve all salts. If iron is present add 20 per cent sodium hydroxide solution to a deep red color. If iron is absent add phenolphthalein and neutralize to the first faint pink. Dilute to volume if an aliquot is to be taken. To the solution, or an aliquot of it, add 2 ml. of a solution containing 15 per cent of concentrated orthophosphoric acid and 15 per cent of concentrated sulfuric acid. Determine with nitroso-R salt as reagent.

Nickel Salts. Weigh out a sample expected to contain about 0.1 mg. of cobalt. This may be several grams of the original salt. Dissolve in water and transfer to a 25-ml. volumetric flask. Add 4 ml. of 1:1 hydrochloric acid. Use an aliquot for determinatiton by extraction as the thiocyanate. The acidity is already properly adjusted for that method.

Unsintered Metal Carbides. Prepare the sample as for determination of iron (page 287) and use an aliquot.

Silicates. Decompose as already described for nickel determination (page 312) up to the point where the first sample solution and the sec-

[6] R. B. Young, E. T. Pinkney and R. Dick, *Ind. Eng. Chem., Anal. Ed.* 18, 474 (1946)

ond sample solution have been obtained. To the first sample solution add 5 ml. of 0.01 per cent solution of dithizone in carbon tetrachloride. Shake for 0.5 minute, let separate, and draw off the extract. Continue to extract with 3-ml. portions of the dithizone solution until the color of the extract is not altered. Similarly extract the second sample solution with 2-ml. portions if there is any cobalt shown by extraction. Combine the extracts and wash with 5 ml. of water. Withdraw the carbon tetrachloride layer into a silica dish, and evaporate to dryness. Rinse down the edges of the dish with carbon tetrachloride and again evaporate. Ash at a low temperature until organic matter is destroyed. Add 0.2 ml. of concentrated hydrochloric acid and 0.2 ml. of concentrated nitric acid to the cooled ash. Evaporate to dryness on a steam bath. Take up in a few ml. of water to which 0.2 ml. of 20 per cent stannous chloride solution in 1:5 hydrochloric acid has been added. Determine by the thiocyanate method.

Soil. A method of preparation of sample has been given for determination of chromium (page 271). In that method a precipitate was set aside for use in determination of cobalt. Transfer the precipitate and paper to a platinum dish and mix with 10 ml. of concentrated nitric acid. Heat until the paper is decomposed and solution of the residue is complete. Let cool and complete as described for copper ores, taking into account that the cobalt content may be lower than expected in that method. Start at "After decomposition is complete, add 5 ml. of 1:1 sulfuric acid . . ."

Biological Samples.[7] Wet-ash according to conventional methods. Finally adjust the solution of ash to approximate neutrality and determine by *o*-nitrocresol.

Alternatively,[8] dry ash at not over 500°. Dissolve 1-2 grams of ash in 5 ml. of 1:100 hydrochloric acid. Without filtering, make the solution alkaline to phenolphthalein with 1 per cent sodium hydroxide solution. Boil for 15 minutes to remove ammonia. Filter the precipitate and wash with 4-5 ml. portions of hot water. Combine the filtrate and washings in a 50-ml. volumetric flask. Acidify with 1:4 hydrochloric acid to a pH of 4-5 and make up to volume.

Foods.[9] Ash a sample containing 0.001-0.01 mg. of cobalt in a muffle

[7] G. H. Ellis and J. F. Thompson, *ibid.* **17**, 254-7 (1945).
[8] C. P. Sideris, *ibid.* 9, 145-7 (1937).
[9] N. D. Sylvester and L. H. Lampitt, *J. Soc. Chem. Ind.* 59, 57-60 (1940).

at 500-550° Cool, add 3 ml. of concentrated sulfuric and 3 ml. of concentrated nitric acids, and return to the muffle. Take up the cooled ash in 10-15 ml. of concentrated hydrochloric acid and evaporate on a hot plate. Cool and dissolve the residue in 25 ml. of 1:4 hydrochloric acid. Filter, dilute to 50 ml., and add 10 ml. of 1 per cent solution of α-nitroso-β-naphthol in glacial acetic acid. Bring to a boil and allow to settle overnight at room temperature. Filter through a pulped filter-paper pad in a Gooch crucible and wash with 10 ml. of 5 per cent acetic acid. Wash the beaker with 2 ml. of warm concentrated sulfuric acid, then with 2 ml. of warm 60 per cent perchloric acid, and reserve these washings to rinse the crucible. Invert the crucible with the precipitate over a Pyrex boiling tube, push a platinum wire through a hole in the crucible to dislodge the pad, and transfer it to the Pyrex tube. Place the crucible upright over the mouth of the tube, and wash with the 2 ml. of warm sulfuric and 2 ml. of warm perchloric acids that have been used to wash the beaker. Carefully heat the contents of the Pyrex tube until a colorless acid residue is obtained. Continue heating until an intense yellow color appears and boil until this color disappears. Cool and dilute with 30 ml. of water. This solution contains copper, iron and cobalt.

Transfer to a separatory funnel and remove copper by adding, with agitation, 5-ml. portions of 0.15 per cent dithizone solution in chloroform, until the last portion of dithizone shows only a clear green color. Drain the dithizone layer, which may be used for determination of copper (page 99). Add 10 ml. of 25 per cent ammonium citrate, made just alkaline with 1:4 ammonium hydroxide. Follow with 1:4 ammonium hydroxide until the small amount of dithizone that remains from the previous extraction becomes orange in color. Add with agitation 5 ml. of 0.15 per cent dithizone solution, separate, and follow with 5 ml. of chloroform. Combine the extracts and evaporate to dryness in a 100-ml. Pyrex beaker. Moisten the residue with 0.5 ml. of concentrated sulfuric acid and 5 drops of 60 per cent perchloric acid. Cover and heat on a hot plate until all organic matter is destroyed. If necessary, add additional perchloric acid. Continue heating uncovered to remove perchloric acid. Dilute to 5 ml. and use as aliquot in the estimation of cobalt, preferably by the nitroso-R-salt method.

Plant Tissue. A solution containing the cobalt and copper will have been isolated by dithizone extraction in determination of lead (page 30) and worked up under copper (page 102). Use an aliquot.

Paint and Varnish. Ash a suitable sample to give about 0.1 gram of ash, and to the cooled ash add 5 ml. of 1:1 hydrochloric acid. If any undissolved residue remains, decant. Add 3 ml. of concentrated hydrochloric acid and 1 ml. of concentrated nitric acid to the insoluble matter. Heat, dilute, filter, and wash any residue of silica. Evaporate the combined extracts to dryness on a water bath. If nitric acid was used, add 2 ml. of concentrated hydrochloric acid and again evaporate to dryness. Dissolve in 25 ml. of 1:10 hydrochloric acid.

If copper is present dilute to 150 ml. and saturate with hydrogen sulfide. After precipitation is complete, filter and concentrate by evaporation to about 25 ml.

If it is necessary to remove nickel, add 10 ml. of 10 per cent ammonium citrate solution to the solution of the sample. Heat and add a slight excess of a 1 per cent alcoholic solution of dimethylglyoxime. Add ammonium hydroxide until the liquid is slightly alkaline. Filter and wash. Evaporate the filtrate to dryness and ignite carefully to destroy excess dimethylglyoxime. Treat the residue with 3 ml. of concentrated hydrochloric acid and 1 ml. of concentrated nitric acid. Evaporate to dryness on the water bath, add 2 ml. of concentrated hydrochloric acid, and again evaporate to dryness. Dissolve in 25 ml. of 1:10 hydrochloric acid.

If the *manganese* is not in excess of the cobalt it may be ignored. If necessary to remove manganese add 25 ml. of 1:1 nitric acid to the solution. Add 0.1 gram of sodium bismuthate. Digest until the permanganate color disappears and the manganese is precipitated. Filter and evaporate the filtrate to dryness on a water bath. Treat with 2 ml. of concentrated hydrochloric acid, again evaporate to dryness, and dissolve the residue in 25 ml. of 1:10 hydrochloric acid. If desirable, dilute further and take an aliquot.

Isolation of Cobalt. In many methods the presence of heavy metals such as copper, nickel, and iron interferes. There are several well-established methods for isolation of the cobalt when the method of determination requires it.

Dithizone Extraction.[10] Extract the cobalt from a properly adjusted solution with dithizone. When heavy metals interfere remove in a preliminary extraction at pH 5.[11] For a more detailed discussion of these

[10] G. H. Ellis and J. F. Thompson, *Ind. Eng. Chem., Anal. Ed.* 17, 254-7 (1945).

[11] Hedley R. Marston and Douglas W. Dewey, *Australian J. Exptl. Biol. Med. Sci.* 18, 343-52 (1940).

reageuts and of precoutions necessary in their preparation refer to page 3.

Transfer the solution of sample to a separatory funnel. For each gram of original sample represented by the solution, add 1 ml. of 40 per cent ammonium citrate solution. Add 2 drops of phenolphthalein solution and adjust the pH to 8.5 with 1:1 ammonium hydroxide. If a precipitate forms, add more ammonium eitrate solution. Add 10 ml. of 0.05 per cent dithizone solution in carbon tetrachloride and shake vigorously for 30 seconds. Draw off the solvent layer and repeat the treatment with dithizone until the carbon tetrachloride phase retains its pure green color. The cobalt is now all in the dithizone extract. Combine the carbon tetrachloride fractions and evaporate to dryness in a beaker on a hot plate.

Add 2 ml. of 60 per cent perchloric acid to the residue and heat the covered beaker on a hot plate at gentle reflux until the solution is colorless. Remove the cover oud evaporate the perchloric acid. Add 5 ml. of 0.01 N hydrochloric acid to dissolve the sample. As described it is designed for estimation of cobalt by *o*-nitrosocresol but is also applicable to the use of other reagents.

Extraction as Diethyldithiocarbamate. Transfer the sample solution to a separatory funnel, and for each gram of original sample add 1 ml. of 40 per cent ammonium citrate solution. Add 2 drops of 0.04 per cent bromothymol blue as an internal indicator and adjust the pH to 6.5 with 1:1 hydrochloric acid or 1:1 ammonium hydroxide. Add, dropwise and with agitation, 5 ml. of 0.1 per cent sodium diethyldithiocarbamate that has been extracted with carbon tetrachloride to remove copper and cobalt. Add 10-20 ml. of carbon tetrachloride and shake vigorously for 10 minutes. Copper and cobalt are quantitotively extracted. Separate and evaporate the carbon tetrochloride extract to dryness in a beaker. Complete as for a dithizone extract starting at "Add 2 ml. of 60 per cent perchloric acid . . ."

Extraction as Thiocyanate.[12] As a 60 per cent reagent dissolve 420 grams of ammonium thiocyanate in 280 ml. of water. Add 1:2 ammonium hydroxide until just alkaline to phenolphthalein. Extract the solution with 10-ml. portions of 0.1 per cent dithizone solution in chloroform as long as the extracts are discolored. Wash the solution with 10 ml. of

[12] N. S. Bayliss and R. W. Pickering, *Ind. Eng. Chem., Anal. Ed.* 18, 446-8 (1946).

chloroform to remove dithizone, then with 10 ml. of amyl alcohol to remove chloroform. Neutralize an aliquot of sample containing 0.01-0.02 mg. of cobalt. Concentrate to less than 20 ml. if necessary. Add 5 ml. of 1:1 hydrochloric acid and 20 ml. of 60 per cent ammonium thiocyanate. Red color is due to iron.

Prepare a molar solution of ammoninm citrate as follows. Dissolve 210 grams of citric acid in 600 ml. of 1:2 ammonium hydroxide. Cool and add ammonium hydroxide until the solution is alkaline to phenolphthalein. Extract interfering metals as has been described for the thiocyanate reagent. Finally dilute to 1 liter. Add the prepared ammonium citrate buffer until the red color disappears. This marks approximately the optimum pH for extraction of the cobalt. Dilute to about 50 ml. and add 4 ml. of ether to saturate the solution. Extract with three 20-ml. portions of 2 ether:1 amyl alcohol. The cobalt is extracted as $(NH_4)_2Co(CNS)_4$ but the iron is retained in the aqueous layer by the buffer. Combine the extracts and discard the aqueous layer.

Transfer the cobalt back to aqueous solution by shaking the extracts with two 20-ml. portions of 1:7 ammonium hydroxide. Discard the solvent layers and evaporate the ammoniacal extracts to dryness. Add 20 ml. of 3:8 nitric acid to the residue and again evaporate to dryness to destroy thiocyanate. Add 1:7 ammonium hydroxide to this residue until it is definitely alkaline and evaporate to dryness. Take up this residue in 5 ml. of water and use for determination by nitroso-R salt.

Separation of Cobalt by α-Nitroso-β-naphthol.[13] The sample should be relatively low in free acidity, neutralized if necessary. The method is suitable for isolation of cobalt from a solution of a steel sample. Precipitate iron and chromium by addition of a 10 per cent dispersion of zinc oxide. Dilute to 200 ml. and filter. To 100 ml. of filtrate add 5 ml. of concentrated hydrochloric acid. Pass in hydrogen sulfide to precipitate copper and filter off the copper sulfide. Boil the filtrate to remove hydrogen sulfide. Add 0.2 gram of α-nitroso-β-naphthol dissolved in 10 ml. of glacial acetic acid. Boil a few minutes and let stand in a warm place for a half hour.

Filter off the precipitate of cobalt-α-nitroso-β-naphthol. Wash with 1:4 hydrochloric acid and with hot water. Ignite in a porcelain crucible in a muffle at about 800°. Dissolve the residue in 10 drops of hot concentrated hydrochloric acid, dilute with 10 ml. of water, and filter if

[13] W. J. Agnew, *Analyst* 53, 31-2 (1928); cf. N. D. Sylvester and L. H. Lampitt, *J. Soc. Chem. Ind.* 59, 57T-60T (1940).

necessary. Depending on the amount of cobalt present and the method to be used, dilute to a known volume and take an aliquot, or use the entire solution.

Removal of Iron as Fluoride. Follow the technic described for copper (page 107).

Standard

Dissolve 0.4936 gram of cobalt nitrate, $Co(NO_3)_2 \cdot 6H_2O$, in water and dilute to 1 liter. Alternatively use 0.263 gram of freshly ignited anhydrous cobalt sulfate in the same final volume. This contains 0.1 mg. of cobalt per ml. Dilute as necessary.

Cobalt by Ammonium Thiocyanate

An estimation of cobalt may be made from the blue color of $(NH_4)_2Co(SCN)_4$, which results on treating the solution with ammonium thiocyanate.[14] The color intensity of the thiocyanate is not high so that a method of intensification is desirable. In addition, it fades within a fairly short time. A high concentration of thiocyanate is essential in the aqueous solution to prevent ionization of the complex which causes some change from blue to the pink of cobalt ion. Therefore it is usual to use a concentration of over 30 per cent of thiocyanate. This also intensifies the color. This may be extracted with amyl alcohol, butyl alcohol,[15] or ether,[16] or intensified by addition of acetone.[17] Solvents are used for extraction of low concentrations of cobalt, whereas the acetone is used for intensification of color in higher concentrations.

Mercury seriously interferes with this method.[18] If iron and nickel are present, pyrophosphate may be used in the proportion of 8 parts of salt to 1 of metal.[19] Insufficient pyrophosphate permits the color to be masked by the red ferric complex. An excess of pyrophosphate inhibits the color due to formation of cobalt pyrophosphate unless there is about 5 times as much thiocyanate as pyrophosphate present. Nickel

[14] H. W. Vogel, *Ber.* 12, 2314 (1879); A. D. Powell, *J. Soc. Chem. Ind.* 36, 273-4 (1917); E. S. Tomula, *Z. anal. Chem.* 83, 6-14 (1931); Harry M. Putsché and W. Francis Malooly, *Anal. Chem.* 19, 236-8 (1947).

[15] N. Foglino and S. Bertoldi, *Ann. chim. applicata* 32, 206-15 (1942).

[16] Josef Babicka, *Mem. soc. roy. sci. Bohême* 1934, No. 11, 1-4 (1935); *Ber. ges. physiol. exptl. Pharmakol.* 88, 401 (1934).

[17] Bendikt Mader, *Die Chemie* 55, 206-7 (1942).

[18] I. F. D. Dwyer, *Australian Chem. Inst., J. and Proc.* 3, 239-44 (1936).

[19] V. M. Zvenigorodskaya, *Zavodskaya Lab.* 7, 1350-5 (1938).

pyrophosphate also has a color but this is discharged with a small amount of ammonia or removed by use of a yellow filter. Sodium acetate and sodium fluoride decolorize iron.[20]

Cobalt is determined in the presence of large quantities of nickel by extraction with various mixtures of isoamyl alcohol and ether, adding ammonium acetate and a few drops of 10 per cent tartaric acid solution after the solvent has been added.[21] For cobalt in steel the method is preferable to the cobalt-ammonia complex or the color in concentrated hydrochloric acid.[22] A few drops of 20 per cent stannous chloride in 1:5 hydrochloric acid will reduce any copper present and prevent interference.[23]

As a modification of this method, if less than 0.1 gram of cobalt per liter is present, Novelli's reagent may be employed. Equal volumes of fresh 0.05 per cent sodium nitrite and resorcinol are mixed, and one-twentieth volume is added to the sample prior to making acid with hydrochloric acid and the addition of ammonium thiocyanate and ether. The red ether layer is compared against standards.[24] Up to 10 times the amount of nickel is permissible.

Chromium, manganese, nickel, zinc, titanium, molybdenum, and uranium do not give colored compounds soluble in ether and amyl alcohol.[25] Vanadium gives a blue complex which is extractable, but addition of ammonium acetate and tartaric acid to the reagents inhibits its formation. The method is applicable for amounts up to 4 per cent by suitable dilution. The minimum amount of cobalt conveniently determined in the aliquot is 0.01 mg. As little as 0.0005 mg. can be detected.[26]

Procedure. *Without Extraction.*[27] Pipet out a sample to contain 0.2-2.0 mg. of cobalt. Adjust the acidity so that it will be about 1:12 with hydrochloric acid. This is done conveniently by neutralizing and adding 8 ml. of 1:1 hydrochloric acid. Add 0.1 ml. of 50 per cent ammonium thiocyanate solution which will give a red color of ferric thiocyanate if iron is present. Add 10 per cent tetrasodium pyrophosphate

[20] V. K. Zemel, *ibid.* 4, 1178-80 (1935).

[21] Yu. Yu. Lur'e and M. I. Troitskaya, *Mikrochemie* 22, 101-8 (1937); G. A. Pevtsov, *Zavodskaya Lab.* 8, No. 10-11, 1176-7 (1939).

[22] Erich Stengel, *Die Chemie* 56, 47-9 (1943).

[23] E. B. Sandell and R. W. Perlich, *Ind. Eng. Chem., Anal. Ed.* 11, 309-11 (1939).

[24] María S. Savari, *Rev. col. farm. nach. Rosario* 6, 159 (1939); *Anales farm. bioquím* (Buenos Aires) 11, 4 (1940).

[25] R. S. Young and A. J. Hall, *Ind. Eng. Chem., Anal. Ed.* 18, 264-6 (1946).

[26] Benedikt Mader, *Die Chemie* 56, 215-18 (1943).

[27] V. M. Zvenigorodskaya, *Zavodskaya Lab.* 7, 1350-5 (1938).

solution dropwise until the red color just disappears, recording the volume added. The solution will be pale green if nickel is present. Add 10 ml. of 50 per cent ammonium thiocyanate solution, and then one-half the volume of pyrophosphate solution previously added. Dilute to 50 ml. and add acetone to a volume of nearly 100 ml. If the solution is greenish, add 1:1 ammonium hydroxide to discharge that color to a clear blue and make to volume with acetone. Compare with standards or read the transmittance at around 600 mμ The color fades within a short time but can be matched for visual comparison by standards of copper sulfate. Photometric reading is preferable.

By Extraction.[28] This method is applicable in the presence of interfering amounts of nickel and iron. Prepare a reagent to contain 125 grams of hydrated sodium thiosulfate and 31.25 grams of hydrated trisodium phosphate per liter. To 8 ml. of this add 10 ml. of 60 per cent ammonium thiocyanate solution and mix. Add 5 ml. of sample solution, which should be around pH 1 and contain 0.01-0.1 mg. of cobalt per ml. Mix and add 7.5 ml. of ether and 2.5 ml. of amyl alcohol. Shake well and, after separation, discard the aqueous layer. Read the color photometrically.

Cobalt by Nitroso-R-Salt

Cobalt forms a red complex with sodium 1-nitroso-2-naphthol-3,6-disulfonate,[29] nitroso-R-salt, which is stable in boiling nitric acid. This last property makes possible a determination that is nearly specific for cobalt. The yellow color of the excess reagent may be completely removed by the addition of bromine,[30] or a blank may be run and correction made. Cobalt combines with 3 mols of the reagent, nickel and copper with one each.[31] Amounts of nickel, copper, and zinc present in biological samples do not interfere.[32] Cyanide and oxidizing or reducing agents should be absent.

[28] R. S. Young and A. J. Hall, *Ind. Eng. Chem., Anal. Ed.* 18, 264-6 (1946).

[29] H. S. Van Klooster, *J. Am Chem Soc.* 43, 746-9 (1921); K. W. McNaught, *Analyst* 64, 23-7 (1939); *ibid.* 67, 97-8 (1942); R. S. Young, E. T. Pinkney and R. Dick, *Ind. Eng. Chem., Anal. Ed.* 18, 474-6 (1946); Nobuyuki Tanaka, *Bull. Chem. Soc. Japan* 18, 436 46 (1943).

[30] Hedley R Marston and Douglas W. Dewey, *Australian J. Exptl. Biol. Med. Sci.* 18, 343-52 (1940).

[31] D P. Malyuga, *Zhur. Anal. Khim.* 1, 176 85 (1946).

[32] N S. Bayliss and R. W. Pickering, *Ind. Eng. Chem., Anal. Ed.* 18, 446-8 (1946).

Copper and large quantities of iron may interfere.[33] Iron may be removed by acidifying the solution with hydrochloric acid and extracting ferric chloride with ether. Both copper and iron are removed by adding sodium acetate and boiling briefly. Alternatively, the iron in the ferric state is precipitated by an excess of ammonium hydroxide. The determination is also sometimes carried out with the iron left in the solution.[34] The sample must be so dilute that nickel is inappreciable. The interfering colors are masked out by a green filter.[35] Copper is precipitated by hydrogen sulfide, which at the same time removes silver, mercury, lead, bismuth, cadmium, arsenic, antimony, tin, molybdenum, selenium, tellurium, gold, platinum, and palladium. Magnesium is separated from cobalt by the use of 8-hydroxyquinoline.[36] A large excess of calcium interferes.[37]

By photoelectric methods the color is read in the presence of iron and nickel.[38] No fading of color was observed in 24 hours' exposure to light but there was appreciable lessening of color in the next 24 hours. The most suitable wave length for reading the transmittance is 420 mμ, or alternatively a blue filter[39] is used.

The method is used[40] for estimation of potassium, by the indirect method of precipitation of potassium cobaltinitrite. Up to 1 per cent of cobalt can be determined with an accuracy of 1 per cent. By suitable dilution or by reduction in the amount of sample taken, higher concentrations can be estimated. Lovibond discs representing the color are available.

Procedure. Transfer a 10-ml. aliquot containing 0.01-0.1 mg. of cobalt. Unless the solution is known to be properly adjusted as to acidity for this determination, evaporate just to dryness. Add 1 ml of concentrated nitric acid and again take to dryness. Take up the sample with 10 ml. of 1:50 hydrochloric acid and add a drop of concentrated nitric

[33] E. B. Kidson, H. O. Askew, and J. K. Dixon, *New Zealand J. Sci. Tech.* 18, 601-7 (1936); E. B. Kidson and H. O. Askew, *ibid.* 21, 178B 89B (1940).

[34] F. W. Haywood and A. A. R. Wood, "Metallurgical Analysis," p. 47-8 (1943); F. W. Haywood and A. A. R. Wood, *J. Soc. Chem. Ind.* 62, 37-9 (1943).

[35] Herbert T. MacPherson and James Stewart, *Biochem. J.* 32, 763-7 (1938).

[36] H. Ronald Fleck and A. M. Ward, *Analyst* 58, 388-95 (1933).

[37] J. W. H. Lugg and S. W. Josland, *Australian J. Exptl. Biol. Med. Sci* 14, 319-21 (1936).

[38] A. Norman Hixson and Wallace M. McNabb, *Metal Finishing* 44, 208-9 (1946).

[39] Hobart H. Willard and Samuel Kaufman, *Anal. Chem.* 19, 505 (1947).

[40] C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* 9, 145-7 (1937).

acid. Heat to boiling to dissolve the salts and cool. Add 5 ml. of 50 per cent sodium acetate trihydrate solution. After mixing, add 10 ml. of 0.2 per cent aqueous nitroso-R-salt solution. Boil for 2 minutes, when the solution should be alkaline to litmus. Filter any precipitate of copper, nickel, and ferrous iron. Filter and wash the precipitate with saturated sodium acetate solution until the washings are colorless. Add 2 ml. of 1:1 nitric acid to the filtrate which should alter it from brownish green to orange red. Boil for 1 minute, cool, and dilute to a known volume to read the transmittance at 420 mμ. Longer boiling will result in reduction of the color intensity, whereas a lesser period does not give full color development.

Cobalt by α-Nitroso-β-Naphthol

When divalent cobalt is treated with α-nitroso-β-naphthol, it is oxidized to the trivalent form and precipitated as cobaltinitroso-beta-naphthol. That compound can be maintained in colloidal suspension for a sufficiently long period of time for duplication, or taken up in chloroform for comparison.[41] The color developed is orange to red and is most satisfactory for photometric reading when a green filter is used. By precipitation of the cobalt, it can be separated from manganese, dissolved in chloroform, and the transmittance read. It has been suggested that the color be developed close to pH 5.2 and that the color of excess reagent be destroyed with sulfite.[42]

If the sample contains iron, aluminum, and chromium, they are kept in solution by addition of ammonium citrate. More than a trace of copper, and any considerable amount of nickel or manganese, must be removed prior to addition of the reagent. The alkalinity of solutions compared should be approximately the same. One ppm. in the solution examined is easily detected. The accuracy should be better than 5 per cent.

Procedure. Prepare a reagent as follows. To 24 ml. of 0.75 per cent sodium hydroxide solution, add 0.1 gram of α-nitroso-β-naphthol and boil. Cool, filter, and dilute to 200 ml. This solution keeps indefinitely.

[41] M. Ilinski and G. von Knorre, *Ber.* 18, 699-704 (1885); *Z. anal. Chem.* 24, 595-8 (1885); F. W. Atack, *J. Soc. Chem. Ind.* 34, 641 (1915); E. G. Jones, *Analyst* 43, 317 (1918); C. Mayr and Fritz Feigl, *Z. anal. Chem.* 90, 15-19 (1932); Louis Waldbauer and Nell M. Ward, *Ind. Eng. Chem., Anal. Ed.* 14, 727-8 (1942).

[42] Ian A. Black, *Soil Sci.* 51, 387-91 (1941).

Duplication. Transfer the sample to a Nessler tube and add 5 ml. of ammonium citrate solution prepared by dissolving 500 grams of citric acid in 250 ml. of water and adding 500 ml. of concentrated ammonium hydroxide. Dilute to 95 ml. with distilled water. Add 5 ml. of reagent and mix. As standard, mix 25 ml. of 1:10 hydrochloric acid, 10 ml. of ammonium citrate solution, and distilled water to make 90 ml. Add 5 ml. of reagent, mix well, and add stock cobalt solution until the color of the sample is duplicated. Adjust the volume to 100 ml.

In Organic Solvent. Prepare an ammonium citrate solution containing 100 grams of citric acid, 25 ml. of water, and 100 ml. of concentrated ammonium hydroxide. To 10 ml. of this, add 1:2 hydrochloric acid until it is just acid. Add the aliquot of sample and mix. Add 25 ml. of reagent, mix, and let stand for 2 hours. The cobalt derivative is quantitatively precipitated. Filter and wash to free the paper and precipitate from excess reagent. Dry at 100°, then extract the precipitate from the paper with successive 10 ml. portions of chloroform until the last extract is colorless. Dilute the extract to 100 ml. and read the transmittance.

Cobalt by β-Nitroso-α-Naphthol

Although the use of the α,β derivative is very old it is only comparatively recently that the greater sensitivity of β-nitroso-α-naphthol has been given consideration for colorimetric estimation of cobalt.[43] As with the α,β compound the color may be read directly in aqueous solution or extracted into an organic solvent.

The maximum sensitivity occurs at a wave length of 550 mμ. The concentration of ammonium hydroxide, which is introduced to delay precipitation of cobalt nitroso-naphtholate, must be closely controlled. An increase in ammonium hydroxide results in a rapid decrease in the absorption of the solution. The presence of ammonium citrate, whose concentration is not as critical, serves as a buffer and to keep traces of iron and other metals from precipitating. The 2-nitroso-1-naphthol-4-sulfonic acid derivative has also been used to determine cobalt[44] at an optimum pH of 7-8, in the presence of 1000 times as much nickel.

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[43] I. Belluci, *Gazz. chim. ital.* **49**, II, 294 8 (1919); Carlos E. Cardini, Walter Jung and Marcos Fuksman, *Anales asoc. quím. argentina* **31**, 191-201 (1943).

[44] L. A. Sarver, *Ind. Eng. Chem., Anal. Ed.* **10**, 378 (1938).

Procedure. *Extraction.*[45] Pipet out an aliquot of sample containing 0.0005-0.1 mg. of cobalt. Neutralize with 10 per cent sodium hydroxide or 1:10 acetic acid to pH 5.7. Dilute or concentrate to about 20 ml. and add 5 ml. of a 1 per cent solution of β-nitroso-α-naphthol in a 40 carbon tetrachloride-60 ethanol mixture. Shake for 5 minutes to form the complex and extract it into the insoluble solvent layer. Discard the aqueous layer and shake the solvent with two 10-ml. portions of concentrated hydrochloric acid. This decomposes and extracts from the solvent layer complexes with iron, copper, and chromium and removes some excess reagent. Discard these acid extracts. Wash the solvent layer successively with 10 ml. of water, 10 ml. of 4 per cent sodium hydroxide, and 10 ml. of water. Discard these extracts and filter the solvent layer through paper. Read the transmittance at 550 mμ and compare with a standard curve.

Direct Determination. As reagent, to 0.1 gram of sodium salt of β-nitroso-α-naphthol,[46] add 20 ml. of water, followed by 1 ml. of 4 per cent sodium hydroxide solution. Heat to dissolve completely and dilute to 200 ml.

Transfer a 10-ml. aliquot of sample containing approximately 0.01 mg. of cobalt[47] to a 100-ml. volumetric flask. Add 5 ml. of ammonium citrate solution containing 500 grams of citric acid in 250 ml. of water, diluted to 1 liter with 1:1 ammonium hydroxide. Add 5 ml. of 1:4 ammonium hydroxide. Dilute the solution to about 90 ml. and add 3 ml. of reagent. Dilute to the mark and mix thoroughly. Read the transmittance against a standard curve or compare directly against a set of freshly prepared standards. The system conforms to Beer's law in the specified range.

Cobalt by o-Nitrosoresorcinol

o-Nitrosoresorcinol has been found to give a more stable, although slightly less sensitive, reaction than β-nitroso-α-naphthol.[48] The stability of the red color extends over a period of several weeks. The color developed is not extractable with organic solvent. It conforms to Beer's law.

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[45] E. Boyland, *Analyst* **71**, 230-1 (1946); cf. Walter Jung, Carlos E. Cardini and Marcos Fuksman, *Anales asoc. quím. argentina* **31**, 122-38, 191-201 (1943).

[46] F. W. Atack, *J. Soc. Chem. Ind.* **34**, 641-3 (1915).

[47] John H. Yoe and Charles J. Barton, *Ind. Eng. Chem., Anal. Ed.* **12**, 405-9 (1940).

[48] Lyle G. Overholser and John H. Yoe, *ibid.* **15**, 310-13 (1943).

327). Shake vigorously for 15-20 seconds and let the layers separate. Remove the brown petroleum-ether layer and save it. Repeat this extraction twice more. Wash the aqueous layer twice with 2-ml. portions of petroleum ether. Dilute the combined petroleum-ether extracts to a known volume. Read the transmittance and apply to a calibration curve.

Cobalt by o-Nitrosocresol

o-Nitrosocresol, 1-methyl-3-hydroxy-4-nitrosobenzene, produces an even more intensely colored complex with cobalt than does nitrosophenol,[50] but the principal lines are in the edge of the ultraviolet.

The complex can be extracted with petroleum ether and read colorimetrically. Interference by moderate amounts of iron is avoided by buffering, larger amounts must be separated. The petroleum ether solution may be diluted or concentrated. Beer's law holds over a wide range, and the complex is stable for several days. Amounts of cobalt in the range 0.00002-0.025 mg. can be determined. No trouble with loss of cobalt by sorption in preparation of the sample is reported, unlike the effect with copper or iron. Problems with contamination in the determination are the usual ones. When the reagent shows some decomposition, as by exposure to direct sunlight, the blank goes up. A reducing agent in the final solution takes care of residual traces of iron.

Reagent. It is necessary to produce the reagent to be used.[51] Dissolve 6 grams of hydroxylamine hydrochloride and 15 grams of cupric chloride in 900 ml. of water, and add 5 ml. of metacresol. The proportions are designed to give the optimum pH for the reaction. Add 15 ml. of 30 per cent hydrogen peroxide with stirring. Allow to stand for 2 hours and add 25 ml. of concentrated hydrochloric acid. Shake with a 25-ml. portion of petroleum ether, then with successive 10-ml. portions until all the reagent is extracted. Wash the yellow petroleum ether solution with three 10-ml. portions of water. Shake with successive 50-ml. portions of 1 per cent cupric acetate solution until the petroleum ether phase is colorless. Filter the approximately 400 ml. of deep red solution of the copper complex and store in the refrigerator. Portions are further purified before use as follows.

Mix 75 ml. of the stock solution with 10 ml. of concentrated hydrochloric acid in a separatory funnel and shake with 300-500 ml. of petroleum ether. Discard the aqueous layer, and wash the solvent layer

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[50] G. H. Ellis and J. F. Thompson, *ibid.* 17, 254-7 (1945).
[51] Oskar Baudisch, *Science* 92, 336 (1940); *J. Am. Chem. Soc.* 63, 622 (1941).

with two 100-ml. portions of 0.01 N hydrochloric acid, then with two 100-ml. portions of water. Prepare a buffer solution of 4 grams of boric acid and 8.6 ml. of 4 per cent sodium hydroxide solution per 200 ml. Shake the solvent layer with 25 ml. of this buffer as reagent. Repeat this extraction until the solvent layer is nearly colorless and discard. Refrigerated, the reagent will keep about a month.

Procedure. If the sample contains substantial amounts of iron, isolate the cobalt by extracting with dithizone or carbamate reagent as described under sample. If large quantities of calcium or phosphorous are present, the latter reagent is advocated.

Prepare a buffer by dissolving 2 grams of boric acid in 100 ml. of water, and adding 2.3 ml. of 4 per cent sodium hydroxide solution. Adjust the pH to 7.7-7.8. Add 5 ml. of this buffer to the sample, which should normally be in 5 ml. of 0.01 N hydrochloric acid. Transfer the sample solution to a 60-ml. separatory funnel and add sodium nitrosocresol reagent dropwise until an orange color forms, and 1 ml. in excess. A pink copper complex may also be present.

Distill 70-90° petroleum ether over dilute, alkaline potassium permanganate, then wash 3 times with distilled water in a separatory funnel. Add 5 ml. of this solvent to the nitrosocresol solution of sample and shake for 5 minutes. Discard the aqueous phase and, to the solvent layer, add 5 ml. of 1 per cent cupric acetate solution. Shake 1 minute and discard the aqueous phase. Wash the solvent layer with 10 ml. of water, and then with 5 ml. of a solution containing 10 grams of hydroxylamine hydrochloride and 9.5 grams of anhydrous sodium acetate in 500 ml. of water. The wash solution should have a pH of 5.0 to 5.2.

The solution in petroleum ether will be almost colorless since most of the light absorption occurs in the near ultraviolet. Transfer the solution to an absorption tube and read photoelectrically, using Corning thickness filters 5860 and 4308. Maximum absorption occurs at 360 mμ. The complex is stable for several days.

Cobalt by Terpyridyl

In the determination of very small quantities of cobalt, ranging from 0.1 to 0.5 ppm., the nitroso-R-salt is not sufficiently sensitive, due to the color of the reagent itself. In this range, terpyridyl forms an orange complex which may be read visually or spectrophotometrically.[52]

[52] M. L. Moss and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 15, 74-5 (1943); *cf.* Gilbert T. Morgan and Francis H. Burstall, *J. Chem. Soc.* 1937, 1649-55.

At higher concentrations, selection is a matter of choice. The color is formed immediately. It is not stable over a period of time and should be compared the same day against freshly prepared standards.

Iron, silver, copper, and nickel, must be separated because of their interference in the method. Zinc and cadmium, if present in large concentrations, also combine with the complex, yielding low results in the cobalt determinations. Titanium, zirconium, and cerium are also sources of interference; and antimony, bismuth, and tin may precipitate. Of the anions, dichromate, cyanide, and vanadate interfere sufficiently to require separation. Beer's law holds for 0.5-50 ppm. The maximum absorption occurs at 445 and 505 mμ. Optimum pH values are not critical, since they range from 2 to 10.

Procedure. Using 1:1 hydrochloric acid, 1:1 ammonium hydroxide, or 20 per cent ammonium acetate solution, adjust the pH of the sample to 2-10. Transfer an aliquot to contain 2-5 mg. of cobalt to a 100-ml. volumetric flask and make up to volume.

Dissolve 0.1 gram of terpyridyl in a minimum of 1:1 hydrochloric acid and make up to 100 ml. Add 5 ml. of this reagent to a 25-ml. aliquot of sample in a 50-ml. volumetric flask, and dilute to volume. Shake thoroughly and take the reading. A blue-green filter such as Corning 428 is suitable. Alternatively compare the color of the developed sample with that of a series of standards prepared on the same day.

Cobalt by 8-Hydroxyquinoline

When a chloroform solution of 8-hydroxyquinoline is added to a solution containing cobalt, an orange complex is extracted into the chloroform layer, which can be read colorimetrically. Complete removal of cobalt is effected in two extractions from an aqueous solution at a pH above 6.8. The chloroform solutions at 420 mμ conform closely to Beer's law up to 10 mg. per liter.[53]

Procedure. Transfer an aliquot of solution to contain about 0.1 mg. per 10 ml. to a separatory funnel. Adjust the pH of the solution to 6.8 using a quinhydrone electrode. Shake vigorously with 4 successive 5-ml. portions of reagent containing 1.4515 grams of 8-hydroxyquinoline per liter of chloroform. Dilute the combined extracts to 50 ml. with chloroform. Read the transmittance at 420 mμ and compare with a calibration curve.

[53] Therald Moeller, *Ind. Eng. Chem., Anal. Ed.* **15**, 346-9 (1943).

Cobalt by Potassium Ferricyanide in Ammoniacal Solution

Cobalt salts give a deep red color with potassium ferricyanide in ammoniacal solution. A linear relationship exists between the color intensity and the cobalt concentration, when a filter covering the 400-470 mμ range is used.[54] The complex formed is stable for one day. Because the reagent itself is colored, the amount must be carefully standardized. Sodium sulfate does not affect the color, but other anions and wide variations in the amounts of ammonium salts do.

Ammoniacal ammonium citrate is added to fix iron prior to addition of the ferricyanide reagent.[55] In general the cobalt must have been separated from other anions but it may have iron precipitated as the basic ferric oxalate in ammoniacal solution.

Procedure. Measure out an aliquot of sample containing 0.5-4.0 mg. of cobalt. This solution must be substantially free of other cations and of nonvolatile anions other than sulfate. Add about 6 ml. of 1:3 sulfuric acid and evaporate to fumes of sulfur trioxide. Cool, dilute, and add 25 per cent sodium hydroxide solution until the first precipitation of cobalt hydroxide is visible. Make the solution just acid with 1:9 sulfuric acid. Dilute, or concentrate by evaporation, to about 50-60 ml. and transfer to a 100-ml. volumetric flask. Add 10 ml. of 1 per cent potassium ferricyanide solution and 20 ml. of concentrated ammonium hydroxide. Dilute to 100 ml., mix, and read the transmittance.

Cobalt as Cobaltammine

If cobalt is oxidized in ammoniacal solution the yellow color changes to an intense rose pink in the course of 1 or 2 minutes. The pink color, which is produced by the cobaltammine is proportional to the amount of cobalt present.[56] Any iron present will be precipitated and may be removed by filtration. The presence of nickel gives rise to a blue color with excess ammonium hydroxide, varying from purple to greenish blue according to the concentrations of ammonium hydroxide and nickel. Manganese is precipitated as manganese dioxide and removed by filtration, or its precipitation may be prevented by the addition of ammonium chloride. One may remove all interfering elements except manganese

[54] R. J. DeGray and E. P. Rittershausen, *ibid.* 14, 858-9 (1942); *ibid.* 15, 26-7 (1943).

[55] G. Bogatski, *Arch. Eisenhüttenw.* 17, 125-6 (1943).

[56] B. S. Evans, *Analyst* 50, 389 (1925).

and nickel by odding a suspension of zinc oxide.[57] The color developed is affected by the concentration of ammonium salts.

Procedure.[58] The sample solution should be neutral or slightly acid. Dilute to obout 40 ml., and add 5 grams of diammonium phosphate, 5 grams of ammonium chloride, and 2 grams of potassium persulfate in 40 ml. of 1:1 ammonium hydroxide. Heat to 80° oad then stir in 2 grams more of persulfate. Boil for 1 minute and let cool. Carefully add concentrated hydrochloric acid to approximate neutrality, dilute to 100 ml., aad let stand overnigbt. Nickel will be precipitated as nickel ammonium phosphate. Therefore as much as 1 gram in the original sample will do no harm. Cobalt remains in solution as tbe chloropentamminecobaltic ion. Read the traasmittaace of the upper layer and compare tbe reading with a curve obtained under similor conditions.

Miscellaneous

The determinatiou of cobalt by the color of the chloride in coaccntrated hydrochloric acid has been used for colorimetric estimatioa,[59] particularly in alloys containiag 0.1-10 per cent of nickel. In this medium, cobalt chloride gives a dark blue color, nickel chloride an intense yellow, and the combinotion of the two a green. The amount of nickel may vary over a considerable range without chaaging the green color produced by a definite weight of cobolt. From 0.2 to 1.9 mg. per ml. can be estimated with an accuracy of 2 per ceut.

The color of the acid solution is destroyed by nitric acid or by free chlorine. Some foreign chlorides, especially those of copper and iron, alter tbe color and must be absent. To prepare the sample evoporote to dryness, take up in concentrated hydrochloric acid, ond again evoporate to dryness, repeating this as long os nitrate or free chlorine remains. Two evaporations are usually sufficient. Finally, take up in concentrated hydrochloric acid and dilute to a known volume.

Prepare stondard solutions of cobalt and nickel containing 2 grams of metal in 1 liter of hydrochloric acid. In one case dissolve 4.9557 grams of nickel nitrate, $Ni(NO_3)_2 \cdot 6H_2O$, and in the otber case 4.9362 groms of cobalt nitrate, $Co(NO_3)_2 \cdot 6H_2O$, in 25 ml. of 1:1 hydrochloric acid. Convert these to the chlorides by several evaporations with 25 ml.

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[57] E. Bischof and G. Geuer, *Angew. Chem.* 54, 238 (1941).

[58] W. R. Schoeller, *Analyst* 69, 8-11 (1944).

[59] C. Hüttner, *Z. anorg. Chem.* 86, 341 (1914); Wilhelm Heinz, *Z. anal. Chem.* 78, 427-39 (1929); H. Pinsl, *Arch. Eisenhüttenw.* 13, 333-6 (1940).

manganese, and iron do not interfere. An amount of nickel double that of the cobalt alters the tint appreciably. Buffer the sample containing about 5 mg. of cobalt as the sulfate to pH 7.5. Add about 10 mg. of cysteine hydrochloride crystals. Gently mix this solution. Oxidation by air will complete the conversion of the cobalt complex to the colored compound. Compare with a standard prepared at the same time from a known amount of cobalt sulfate dissolved in the properly buffered solution.

Oxidation of a cobalt solution by hydrogen peroxide, in the presence of potassium or ammonium bicarbonate, results in the formation of a green hydrocarbonate,[66] $Co(OH)_4 \cdot (COOH)_2$. The method has been applied to indirect estimation of potassium precipitated as cobaltinitrite. The reaction is specific for cobalt and will permit the determination of 4 mg. of cobalt per liter. If the solution is more concentrated, precipitation occurs. In dilute solution no change occurs on standing for weeks. Alkali chlorides up to 80 grams per liter do not affect the color.

Put 10 ml. of sample solution in a 50-ml. volumetric flask. Add 0.5 ml. of 3 per cent hydrogen peroxide and dilute nearly to the mark with a saturated solution of potassium bicarbonate or ammonium bicarbonate. Mix well and complete the dilution with bicarbonate solution or with distilled water. Mix and compare with a standard similarly treated.

Cobalt can be detected by reduction of arsenophosphotungstic acid in 300-400 times its weight of nickel, and 0.25-0.50 mg. estimated in the presence of 2.5-5.0 mg. of nickel. The reaction gives a blue reduction product of tungstic acid in the presence of cyanide.[67] To prepare the reagent, dissolve 100 grams of sodium tungstate in 600 ml. of water. Add 50 grams of pure arsenic oxide, 25 ml. of concentrated orthophosphoric acid, and 20 ml. of concentrated hydrochloric acid. The latter serves as a condensing agent. Boil for 20 minutes, cool, and dilute to 1 liter. The reagent keeps indefinitely.

Add 3 ml. of the reagent to 10 ml. of approximately neutral solution of the sample and to a corresponding standard. Invert both tubes once to mix and add to each 4 ml. of a 5 per cent solution of sodium cyanide containing 2 ml. of concentrated ammonium hydroxide per liter. Invert the two tubes simultaneously and compare between 2 and 10 minutes after mixing. No turbidity will appear within 10 minutes if not over

[66] A. Blanchetière and J. M. Pirlot, *Compt. rend. soc. biol.* **101**, 858-60 (1929).
[67] Abraham Lieberson, *J. Am. Chem. Soc.* **52**, 464-5 (1930).

CHAPTER 20

MANGANESE

Manganese is associated with iron in many minerals. It is a common ingredient of alloys as well as of iron and steel. It is present in small amounts in many biological samples. Aside from that it is an important paint drier and must be substantially absent from anything that will be in contact with rubber since manganese accelerates the deterioration.

The major method of determination of manganese is as the permanganate. The most common oxidizing agents are persulfate and periodate, but even bismuthate and lead dioxide are still used, particularly with iron and steel samples.

If the concentration of permanganate formed is insufficient to be suitable for titration, it is determined colorimetrically. Thus some colorimetric methods are simple modifications of other methods.

Samples

Magnesium Alloys.[1] If the manganese content is under 0.5 per cent, weigh out a sample to contain 0.5-2 mg. If the manganese content is over 0.5 per cent, use a sample containing 10-15 mg. and aliquot later. In either case add 25 ml. of cold water to the sample and slowly add 25 ml. of 1:4 sulfuric acid per gram of sample. When reaction is complete, add 25 ml. of concentrated nitric acid and heat to boiling to dissolve any dark residue. Filter if the solution is turbid. Use the entire solution or an aliquot, adding 5 ml. of 85 per cent orthophosphoric acid and applying the periodate method.

Aluminum Alloys. *Acid Solution.*[2] To 0.5 gram of sample, add 10 ml. of cold 1:10 sulfuric acid. When reaction ceases, heat gently. Copper will remain undissolved. Cool and filter into a 100-ml. volumetric flask. Dilute to volume and use a suitable aliquot as sample.

Alkali Separation.[3] Dissolve a 0.5-gram sample in 10 ml. of 20 per

[1] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 322-3. American Society for Testing Materials, Philadelphia, Pa. (1946).

[2] Herm. A. J. Stelljes and P. Langer, *Aluminium* 24, 169-72 (1942).

[3] Hans Pinsl, *Aluminium* 19, 439-46 (1937); *ibid.* 20, 706-14 (1938).

cent sodium hydroxide solution. When reaction is complete, the manganese remains in the residue. Filter and wash the residue well. Discard the filtrate and dissolve the residue in 5 ml. of hot 1:10 nitric acid. Dilute to a known volume and aliquot, or dilute to about 25 ml. and use the entire solution as sample.

Combined Method.[4] Dissolve a 0.5-gram sample in 10 ml. of 20 per cent sodium hydroxide solution. When reaction is complete, add 20 ml. of 1:1 nitric acid and boil until solution is complete. Expel nitrous fumes, make up to a suitable volume, and use an aliquot as sample by the persulfate method.

Zinc-aluminum-iron Alloys and Zinc-aluminum-copper Alloys. The sample solution was prepared for determination of copper (page 81). Use an aliquot.

Aluminum-copper-nickel-manganese-iron Alloys. The sample solution was prepared for copper determination (page 80). Use the periodate method.

Iron and Steel. *Cast and Pig Iron.*[5] Transfer a 1-gram sample to a 250-ml. beaker, add 30 ml. of 1:3 nitric acid, and heat until solution is complete. Filter the graphitic carbon and silica that remain, catching the filtrate in a 100-ml. volumetric flask. Wash the filter with hot water and dilute the filtrate to volume. Use the full sample to determine manganese, preferably by the persulfate method.

Carbon Steel. Dissolve a 1-gram sample in 30 ml. of a mixture containing 100 ml. of concentrated sulfuric acid, 125 ml. of 85 per cent orthophosphoric acid, 250 ml. of concentrated nitric acid, and 525 ml. of water. When dissolved, dilute to a suitable volume and determine by the periodate method.

Provision is often made for preoxidation. Thus to 1.0 gram of steel,[6] add 70 ml. of 1:3 nitric acid and boil for 1 minute. Cool and add 1 gram of ammonium persulfate. Boil for 15 minutes to oxidize any carbon

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[4] F. Pavelka and Hermine Morth, *Mikrochemie* 13, 305-12 (1933); A. Jordy, *Aluminium* 21, 27-31 (1939).

[5] W. M. Murray, Jr. and S. E. Q. Ashley, *Ind. Eng. Chem., Anal. Ed.* 10, 1-5 (1938); R. W. Silverthorn and J. Alfred Curtis, *Metals and Alloys* 15, 245-8 (1942).

[6] Frank W. Scott, *Chemist Analyst* 27, 28-33, 52-7 (1938); cf. N. M. Miloslavskii and E. G. Valislova, *Zavodskaya Lab.* 5, 12-16 (1936).

If the sample contains chromium, the yellow color of chromate ion will modify the color of the final solution. To separate manganese from chromium,[14] render the acid solution just alkaline with ammonium hydroxide and warm for 5 minutes. This precipitates the manganese and iron as hydroxides. Filter and wash thoroughly. Dissolve the manganese and iron from the filter in 1:5 sulfuric acid with the addition of sulfurous acid or hydrogen peroxide and dilute to volume.

Iron Ore. Heat a 1-gram sample with 10 ml. of concentrated hydrochloric acid, replacing the acid evaporated until solution of all attackable material is complete. Filter and wash the paper. Ash the residue and treat with 1 ml. of 1:1 sulfuric acid and 2 ml. of 48 per cent hydrofluoric acid. If the silica content is very high, increase the hydrofluoric acid. Heat gently at first, then strongly, until fumes of sulfur trioxide are given off. Let cool, take up with 10 ml. of water, and filter if necessary. Combine the two solutions as the sample, or take to a known volume and use an aliquot. Apply the periodate method, being sure that sufficient orthophosphoric acid is added to decolorize the solution.

Cyanide Solution of Ore. To 100 ml. of the cyanide solution add 10 ml. of concentrated nitric acid under an efficient hood. Boil until all cyanide is expelled, cool, and dilute to 100 ml. The method was originally designed for lead peroxide oxidation, but, by taking the other metals known to be present into consideration, other oxidizing agents may be used.

Silicates. Mix a 1-gram sample in platinum with 5 ml. of 1:1 sulfuric acid and 5 ml. of 48 per cent hydrofluoric acid. Heat slowly at first, finally to sulfur trioxide fumes. When cool, wash down the sides of the crucible with 5 ml. of 1:5 sulfuric acid. Add 0.2 ml. of 1:1 nitric acid and heat to sulfur trioxide fumes. Let cool and take up in 15 ml. of warm water. Filter and wash to give a volume of about 40 ml. Use the periodate method. The persulfate method is preferred if the silicate is Portland cement.[15]

Minerals. Grind the sample to pass a 150-mesh screen. Mix 1 gram of sample with 4 grams of sodium carbonate and fuse in a platinum crucible until the melt is clear, usually about 10 minutes. Rotate the fusion to form a thin film on the sides of the crucible. Heat the crucible

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[14] M. Dittrich, *Z. anorg. Chem.* **80**, 171 (1913).
[15] E. I. Nagerova and A. D. Lebedeva, *Zavodskaya Lab.* **8**, 1069-73 (1939).

in about 100 ml. of water on the water bath until the melt is disintegrated. Remove the crucible and wash. Acidify the mixture with 130 ml. of 1:5 sulfuric acid and dilute to 250 ml. If a precipitate of silica remains, filter through a dry filter. The silica usually produces only an opalescence. The color may he estimated in the presence of this if the opalescence is slight.

Muds.[16] Dry the sample at 100° for 48 hours and grind to a fine powder. Weigh 0.5 gram into a platinum crucible. Add 5 ml. of 48 per cent hydrofluoric acid and 5 ml. of concentrated sulfuric acid, and evaporate to fumes of sulfur trioxide. Let cool and repeat the addition of 5 ml. of 48 per cent hydrofluoric acid. Repeat further if necessary until the sample is entirely decomposed. Expel the hydrofluoric acid by heating to sulfur trioxide fumes. Drive off the excess sulfuric acid by further heating and fuse the resulting salts. Raise the temperature for 5 minutes, then let tho mass cool. Dissolve in 1:10 sulfuric acid and make up to volume in a 1-liter calibrated flask with 1:10 sulfurio acid for the use of aliquots. The periodate method is suggested.

Soils.[17] Weigh 1 gram of soil into a platinum crucible and ignite at 500° in a muffle to destroy organic matter. Cool, add 25 ml. of 48 per cent hydrofluoric acid and 5 ml. of concentrated sulfuric acid, and digest on a hot plate until white fumes appear. Cool, dilute with 25 ml. of water, and warm to dissolve the salts. Filter the solution and use as sample by the persulfate method. Soils in the United States usually contain 0.05-0.06 per cent of manganese.

Alternatively,[18] stir a 5-gram sample of soil with 50 ml. of 1/40 sulfuric acid for 30 minutes. Heat on a water bath for 30 minutes and filter. Evaporate a 25-ml. aliquot of the filtrate to dryness on a water hath. Cool, add 10 ml. of concentrated nitric acid, and evaporate to dryness. Wash the salts into a 100-ml. volumetric flask and add 5 ml. of 10 per cent silver nitrate. If a precipitate of silver chloride forms, filter. Dilute to volume and use 25-ml. aliquots, preferably employing the persulfate method [19] for which the catalyst is already present. Sodium acetate-acetic acid extracts of soil are also used for estimation of soluble manganese.[20]

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[16] T. G Thompson and T. L. Wilson, *J. Am. Chem. Soc.* **57**, 233-6 (1935).
[17] George J. Hough, *Ind. Eng. Chem., Anal. Ed.* **7**, 408 9 (1935).
[18] P. A. Vlasyuk and V. Ya. Gornaya, *Pedology* (U.S S R.) 1943, No. 9-10, 75-6.
[19] V. T. Illiminskaya, *Problems Soviet Soil Sci. Symposium*, **1**, 149 65 (1936).
[20] Michael Peech and Leah English, *Soil Sci.* **57**, 167-95 (1944).

Sodium Hydroxide and Cell Liquor.[21] The amount of manganese in sodium hydroxide solution is ordinarily less than 1 ppm. Therefore, isolation of the manganese is desirable.

To a sample containing 50 grams of sodium hydroxide, add water to make up to 600 ml. Add 1 ml. of 10 per cent sodium sulfite solution and about 90 ml. of concentrated hydrochloric acid. Then add 3-5 drops of phenolphthalein indicator and the concentrated acid to neutrality, followed by a 5-ml. excess. At this point start a blank of 600 ml. of water with 5 ml. of concentrated hydrochloric acid, which is to be treated thereafter the same as the sample. Add 5 ml. of a 1 per cent solution of 8-hydroxyquinoline and mix well, then stir in 15 ml. of concentrated ammonium hydroxide. Cool the solution to room temperature and transfer to a 1-liter separatory funnel. Rinse the beaker into the funnel with water.

Extract by shaking with 30 ml. of chloroform for 60 seconds and draw off the chloroform layer into a flask. Trichloroethylene or carbon tetrachloride may be substituted. Again extract with 10 ml. of chloroform for 15 seconds and draw this off. Extract with further 10-ml. portions of chloroform until no color is extracted. Add 5 ml. of 70 per cent perchloric acid and 5 ml. of 30 per cent hydrogen peroxide to the chloroform extracts, cover, and evaporate slowly to 1-2 ml. Do not evaporate to dryness as a mild explosion may result. No modification which may increase the amount of organic matter should be made. Cool and rinse off the cover glass. Add 10 ml. of 50 per cent solution of sodium dihydrogen orthophosphate monohydrate and dilute moderately with water for use as sample by the periodate method. Boiling longer than usual is required.

As a simplified method[22] weigh 10 grams of caustic soda and dilute to about a 10 per cent solution. Add 50 ml. of 1:1 orthophosphoric acid and use as sample by the potassium periodate method. To develop the color it is only necessary to add 0.8 gram of reagent and boil for about 25 minutes.

Phosphoric Acid.[23] Dilute 5-10 grams of the acid with water, add 20 ml. of concentrated nitric acid, and heat to boiling. Add sufficient 1 per cent silver nitrate solution to precipitate all chlorides, and 3 ml. in excess. Boil to coagulate the precipitate, cool, and dilute to a definite

[21] Dwight Williams and R. V. Andes, *Ind. Eng. Chem., Anal. Ed.* 17, 28-31 (1945).
[22] Raymond F. Moran and Allen P. McCue, *ibid.* 18, 556-7 (1946).
[23] W. H. Ross, C. B. Durgin and R. M. Jones, *Ind. Eng. Chem.* 14, 535 (1922).

volume. Use an aliquot of the supernatant liquid as sample by the persulfate method.

Water.[24] Oxidation with periodate or with persulfate is the standard method.[25] To 100 ml. of water or a sample containing less than 1 mg. of manganese, add 10 ml. of 1:3 nitric acid and 1 ml. of concentrated sulfuric acid. Evaporate until most of the sulfuric acid is driven off. This destroys organic matter and drives off chlorides. By not taking to dryness the manganese sulfate is easily soluble. Only a small amount of sulfuric acid should be left as it modifies the color. Unless all organic matter is destroyed, low results will be obtained. Cool, take up with 50 ml. of water and 20 ml. of 1:3 nitric acid through which air has been bubbled to remove oxides of nitrogen, and use as sample.

For traces of manganese,[26] raise the pH of the water sample to 9. Add 2 ml. of saturated magnesium sulfate solution per 100 ml., mix well, and let stand. Decant or filter the precipitated magnesium hydroxide and dissolve in about 1 ml. of 1:3 sulfuric acid per 10 mg. of precipitate for use as sample.

Suspended impurities containing manganese are coagulated with potash alum for differentiating from dissolved manganese.

Sea Water.[27] Evaporate a liter sample of filtered sea water to dryness. Add 30 ml. of concentrated sulfuric acid and heat until the fumes of sulfur trioxide appear. Transfer to a platinum crucible and heat carefully to expel all excess sulfuric acid. Heat the fused salts that remain in the crucible over a Meker burner for 5 minutes. Cool, dissolve in 50 ml. of 1:16 sulfuric acid, and bring to the boiling point. Filter through asbestos into a 100-ml. volumetric flask and make nearly to volume with 1:16 sulfuric acid. It is recommended that the entire volume be used as sample and that the periodate method be used.

Plant Tissue and Biological Samples.[28] *Dry Ashing.* Ash a 5.0-gram sample in a muffle at 500°. Cool and add to the ash 10 ml. of 48 per cent hydrofluoric acid. This will prevent loss of manganese as insoluble silicate. Warm gently to dissolve, cool slightly, and add 10 ml.

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[24] W. D. Collins and Margaret D. Foster, *ibid.* **16**, 586 (1924).

[25] American Public Health Association, "Standard Methods of Water Analysis," 9th ed., pp. 56 7 (1947).

[26] V. T. Chuĭko, *J. Applied Chem* (U S S.R.) **11**, 530-3 (1938).

[27] T. G Thompson and T. L. Wilson, *J. Am Chem. Soc.* **57**, 233-6 (1935).

[28] G. W. Leeper, *Proc. Roy. Soc Victoria* **47**, Pt. II, 225-61 (May 8, 1935).

of concentrated sulfuric acid. Evaporate to fumes of sulfur trioxide. Transfer quantitatively to a 100-ml. volumetric flask and dilute to volume. Develop the color with persulfate.[29] The ash may also be taken up directly in 10 ml. of concentrated nitric acid and diluted to volume.[30]

Wet Ashing.[31] Weigh 3-4 grams into a Kjeldahl flask and add 40 ml. of concentrated nitric acid. Place in the hole of a protective asbestos board to avoid heating above the acid layer and boil gently over a gas flame. When nearly dry, and with solid particles completely disintegrated, remove and let cool. With oily materials a second addition of nitric acid is often necessary. Add 13 ml. of 60 per cent perchloric acid at one time and again boil gently. When the nitric acid and water have been evaporated, there will be a vigorous evolution of gas, the solution will boil rapidly, and white fumes will appear. Just before white fumes appear, lower the gas so that the contents will simmer gently for 10-15 minutes Let cool for 5-10 minutes and add a few ml. of water. Filter on asbestos which has been digested with potassium permanganate and acid-washed, or through a sintered glass filter. Wash the filter and use the entire filtrate as sample. The periodate method is preferred.[32]

In the separation of metals in plant ash (page 30) solution A is suitable for direct determination of manganese as well as iron, molybdenum, and phosphorus.

Urine.[33] To 100 ml. of urine in a Kjeldahl flask, or more if the quantity of manganese is less than 1 ppm., add 20 ml. of concentrated nitric acid. Evaporate to a paste on a sand bath. Let cool, add 5 ml. of concentrated sulfuric acid, and heat until about a third is driven off as sulfur trioxide. More sulfuric acid may be needed if phosphates are high or the quantity of sample is large. Cool, add 5 ml. of concentrated nitric acid and heat until the brown fumes disappear. Repeat until oxidation is complete. Add distilled water and 5 ml. of concentrated nitric acid and dilute nearly to 100 ml. for use as sample. If necessary, filter off silica resulting from the attack on the glassware by phosphoric acid.

[29] O. Braadlie and H. Beigh, *Tids. Kjemi. Bergvesen Met.* **2**, 88 9 (1942).

[30] P. A. Vlasyuk and V. Ya. Gornaya, *Compt. rend. acad. sci. U. R. S. S.* **28**, 124-6 (1940).

[31] J. W. Cook, *Ind. Eng. Chem., Anal. Ed.* **13**, 48-50 (1941).

[32] John B. Smith, *J. Assoc. Official Agr. Chem.* **22**, 673-6 (1939).

[33] R. F. McCrackan and E. Passamaneck, *Arch. Path. Lab. Med.* **1**, 586 (1926).

Feces.[34] Ash a 25-gram sample in a muffle at dull red heat. Dissolve the carbon-free ash in 5 ml. of 1:1 hydrochloric acid on the water bath. Let cool, add 2 ml. of concentrated sulfuric acid, and evaporate to sulfur trioxide fumes. Take up in water and dilute to about 25 ml. without filtering. Use the persulfate method.

Fatty Samples.[35] To the sample of 10-100 grams add 25 ml. of concentrated sulfuric acid and heat to boiling for 5 minutes. This separates water from fat. When cool, add 30 ml. of concentrated nitric acid and 10 ml. of concentrated hydrochloric acid. Let stand overnight. Separate fat which has risen to the surface and boil off the excess acid to give a few ml. of clear liquid containing the manganese as sulfate.

Butter.[36] Transfer a 5-gram sample to a 25-ml. porcelain dish and half melt it over an open flame. Place a wick made of 9-cm. filter paper in the dish with both ends protruding over the edge of the dish. Burn the butter slowly, then complete the ashing at 800°. To the ash, add 0.25 gram of sodium carbonate and 0.25 gram of potassium carbonate, and form a melt by heating. Dissolve the melt in 3 ml. of water and transfer to a centrifuge tube. Wash the porcelain dish with 1 ml. of water, then add this to the contents of the centrifuge tube. Centrifuge at 3000 rpm. for 10 minutes. Acidify the clear decantate with a suitable acid according to the method of development of color to be used, dilute to a known volume, and aliquot as necessary.

Fertilizers.[37] Transfer 1 gram of sample to a 200-ml. flask and add 10 ml. of concentrated sulfuric acid and 30 ml. of concentrated nitric acid. Digest on a hot plate near the boiling point. Evaporate to fumes of sulfur trioxide, cool, and add 35 ml. of 1:6 orthophosphoric acid. Bring to a boil and filter into a 200-ml. volumetric flask. Wash the filter with water, collecting the washings in the volumetric flask, and dilute to volume. Use an aliquot according to the manganese content and determine by the periodate method.

Drug Preparations.[38] Weigh out a sample to contain 0.1-1.0 mg. of manganese. Add 5-10 ml. of concentrated sulfuric acid and digest on a

[34] M. M. Retortillo and J. D. Gallego, *Rev. sanidad* **11**, 85-103 (1936).
[35] C. K. Reimann and A. S. Minot, *J. Biol. Chem.* **42**, 329-46 (1920).
[36] G. Schwarz, O. Fischer and B. Hagemann, *Deut. Molkerei-Ztg.* **64**, 143-4 (1943).
[37] John B. Smith and E. J. Deszyck, *J. Assoc. Official Agr. Chem.* **22**, 270-80 (1939).
[38] Elemér Schulek and Pál Menyhárth, *Magyar Gyógyszerésztud. Társaság Ertesitöje* **15**, 513-20 (1939).

steam bath. Repeatedly fume to sulfur trioxide, cool, and cautiously add small portions of hydrogen peroxide to destroy all organic matter. Cool, dilute, filter into a 100-ml. volumetric flask, and make up to volume for determination by the persulfate method. If the iron content is high, the addition of orthophosphoric acid is advisable, and in some cases filtration may be necessary.

For the analysis of liquid preparations such as peptonates, syrup of hypophosphites, and similar products containing 0.05-0.25 mg. of manganese per ml., transfer 5 ml. of the sample solution to a small beaker and dilute to about 100 ml. Add 2-3 ml. of ether and excess ammonium sulfide solution. Heat on the water bath with stirring. The ether causes the sulfides to settle rapidly. After the precipitate settles, filter and wash thoroughly with water containing a little ammonium sulfide. Dissolve the precipitate on the filter with the smallest possible quantity of 1:5 sulfuric acid. Boil to expel hydrogen sulfide and add 1 ml. of 1:3 nitric acid and 1 drop of 2 per cent silver nitrate solution. If this causes turbidity, indicating imperfect washing of sulfides, add a very slight excess of 2 per cent silver nitrate solution, boil again, and filter. The method is applicable in the presence of 3-5 times as much iron as manganese, with persulfate oxidation.

Textiles. Follow the preparation of sample as for copper (page 105) until rendered just acid to Congo red. Add 5 ml. of 10 per cent sulfuric acid and dilute to 200 ml. As a solution for preparation of standards, add 2.5 grams of potassium sulfate, 2.5 grams of sodium sulfate, and 10 grams of ammonium sulfate to water. Dissolve, add 5 ml. of 10 per cent sulfuric acid, and dilute to 200 ml. Use this in parallel with the sample.

Separation from Cobalt.[39] Dilute an aliquot of sample to about 65 ml. and add 4-5 grams of ammonium chloride and 25 ml. of concentrated ammonium hydroxide. Heat nearly to boiling and add 10 ml. of saturated ammonium orthophosphate solution, dropwise, with stirring.[40] Cool the solution and add 10 ml. of 95 per cent ethanol. Manganese ammonium phosphate precipitates in crystalline form. When a proper crystal size has developed, filter and wash with a solution containing 250 ml. of concentrated ammonium hydroxide, 100 ml. of saturated disodium orthophosphate solution, and 100 ml. of 95 per cent ethanol per

[39] Louis Waldbauer and Nell M. Ward, *Ind. Eng. Chem., Anal. Ed.* 14, 727-8 (1942).

[40] V. M. Peshkova and A. A. Ovsyannikova, *Zavodskaya Lab.* 6, 800-3 (1937).

liter. If the precipitate is colored by cobalt, dissolve in 1:2 hydrochloric acid and reprecipitate. Dissolve the phosphate precipitate of manganese in such volume of 1:2 hydrochloric acid as is required, wash the paper well with water, and dilute to a known volume.

Precipitation of Manganese.[41] A standard technic for separation of manganese from cobalt, nickel, zinc, and the rare elements is as follows: Evaporate the material in hydrochloric acid solution almost to dryness. Add 2 ml. of 1:1 nitric acid and evaporate to 1 ml. to remove hydrochloric acid. Rinse the residue into a flask with 10 ml. of 1:1 nitric acid. Place the flask in a beaker of boiling water and very gradually add about 0.5 gram of finely powdered potassium chlorate. Continue heating for about 2 minutes. A black precipitate indicates the presence of manganese. Add additional amounts of potassium chlorate until no further precipitation takes place, but do not add more than 3 grams. Filter with suction through asbestos. Titanium, vanadium, or zirconium, if present, will be carried down with the manganese. Wash the precipitate well with hot water and discard the filtrate and washings.

Dissolve the precipitate from the crucible by heating in 10 ml. of 1:10 sulfuric acid and adding 30 per cent hydrogen peroxide dropwise. Boil to decompose excess peroxide, dilute to a known volume, and use an aliquot.

Separation of Iron.[42] To a neutralized sample add excess of a suspension of zinc oxide, prepared by precipitation of 10 per cent zinc sulfate solution with alkali. Shake, filter, and wash the precipitate with water. Use the filtrate as sample.

Removal of Iron as Fluoride. This has been described for separation from copper (page 107). Modify for separation from manganese by use of 3 ml. of concentrated sulfuric acid per 100 ml. of sample.

Standards

Dilute a recently standardized potassium permanganate solution to contain 0.2877 gram of the salt per liter. Each ml. corresponds to 0.1 mg. of manganese.

For use in reduced form acidify with 1:1 sulfuric acid and slowly

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[41] A. A. Noyes and W. C. Bray, "Qualitative Analysis of the Rare Elements," p 197, Macmillan Co., New York, N. Y. (1927)

[42] L. C. E. Kniphorst, *Chem. Weekblad* 42, 311-16 (1946).

add enough dilute oxalic acid solution[13] to discharge the color before diluting to volume. This results in a standard manganous sulfate solution which is given the same treatment as the solution of sample.

To obtain a standard of the same concentration, dissolve 0.3077 gram of manganese sulfate monohydrate or 0.4061 gram of manganese sulfate tetrahydrate in water, add 1 ml. of concentrated sulfuric acid, and dilute to 1 liter.

Manganese as Permanganate

Manganese is determined by the oxidation of manganese salts to permanganate and comparison of the purple to violet color produced with that of a standard permangate solution. Studies of absorption curves of dilute permanganate solutions indicate absorption maxima at 490, 508, 526, 546 and 566 mμ.[44] Further dilution does not cause any displacement of the bands, nor does Beer's law cease to be valid. The presence of copper in concentrations up to 5 per cent has no influence on measurements.[45] The colors due to nickel and cobalt interfere, regardless of the method of oxidation.

Many different agents have been used to oxidize the manganous ion. Originally, lead dioxide in strong nitric acid solution was the oxidizing agent. This has been recommended especially for the examination of grasses, cereals, and cotton plants.[46] However, filtration or sedimentation is necessary to remove excess lead dioxide. Bismuth tetroxide has been used. The use of sodium hypochlorite[47] for oxidation in the presence of a trace of copper sulfate has been suggested. Oxidation in alkaline solution has been recommended where the yellow color of iron interferes with the permanganate color.[48]

Low concentrations of manganese in chloride solution have been precipitated, with hydrated ferric oxide as a collector, by sodium hydroxide.[49] The precipitate is then dissolved in sulfuric acid. In this way, 0.2

[43] American Public Health Association, "Standard Methods of Water Analysis," 9th ed. p. 57 (1946); cf. M. M. Retortillo and J. D. Gallego, *Rev. sanidad* 11, 85-103 (1936).

[44] B. Lange and C. Schusterius, *Z. physik. Chem.* A159, 295-302 (1932); J. P. Mehlig, *Ind. Eng. Chem., Anal. Ed.* 7, 27-9 (1935); George P. Rowland, Jr., *ibid.* 11, 442-5 (1939).

[45] F. Simgaglia and M. Monticelli, *Alluminio* 9, 83-8 (1940).

[46] A. V. Varadaraja Iyengar, *Indian. J. Agr. Sci.* 8, 819-28 (1938).

[47] G. Denigès, *Gaz. hebd. sci. méd. Bordeaux* 11, 173 (1936); *Rev. sud-americana endocrinol., immunol., quimioterap.* 20, 624.

[48] Hans Pinsl, *Aluminium* 19, 439-46 (1937).

[49] R. Gilbert, *Analyst* 66, 450 (1941).

ppm. of manganese in potassium chloride can be estimated. Perchloric acid, if used with caution, may be employed as an effective oxidizing agent, following wet oxidation of the sample with nitric and sulfuric acids.[50] The optimum concentration for the reagent ranges from 10-15 per cent.

Sodium bismuthate as oxidizing agent yields accurate results with minute amounts of manganese, but the excess reagent must be removed before comparison can be made. The liberation of gas bubbles on the walls of the comparison tube or cell, which is at times evident in solutions oxidized with persulfate, is avoided.[51] No secondary color reaction with other components of the solution occurs. The method gives low results when applied to plant materials,[52] but it is applicable in analysis of manganese ores.[53]

Orthophosphoric acid is not a satisfactory decolorizing agent for iron if the bismuthate reagent is to be used, nor does it decolorize chromium, nickel or cobalt.[54] Both the lead dioxide and the bismuthate methods have been largely replaced by the periodate and persulfate methods.

A combination of the persulfate and bismuthate methods for the complete separation of manganese has been recommended.[55] Hydrated manganese dioxide is precipitated with a large excess of ammonium persulfate in dilute sulfuric acid and the filtered precipitate dissolved by reducing with sodium bisulfite in nitric acid. The manganese is then oxidized to permanganate with sodium bismuthate in the cold. Zinc, chromium, nickel, cobalt, and moderate amounts of tungsten, molybdenum, or phosphorus do not interfere

The permanganate color produced by the persulfate reagent is more fugitive than that developed by the periodate reagent. Silver is a necessary catalyst for the persulfate reaction to prevent precipitation of manganese oxide.[56] This oxidation is effected at 60-70°, and it is believed that the silver forms a peroxide which is an intermediate in the oxida-

[50] J. W. Cook, *Ind. Eng. Chem., Anal. Ed.* **13**, 48-50 (1941).

[51] Erich Bischof and Georg Geuer, *Metall u. Erz* **41**, 57-63 (1944).

[52] Jehiel Davidson and Ruth G. Capen, *J. Assoc. Official Agr. Chem.* **14**, 547-51 (1931).

[53] Antonio Furia, *Rev. brasil chim.* (São Paulo) **9**, 207-8 (1939); *ibid.* **10**, 17-18 (1940).

[54] G. E. F. Lundell, J. I. Hoffman, H. A. Bright, "Chemical Analysis of Iron and Steel," p. 192. John Wiley & Sons, New York, N. Y. (1931).

[55] J. P. Mehlig, *Ind. Eng. Chem., Anal. Ed.* **7**, 27 9 (1935); cf. Ralph H. Müller and J. P. Mehlig, *ibid* 361-2 (1935).

[56] F. Nöthlich, *Intern. Rev. ges. Hydrobiol. Hydrog.* **36**, 562 (1938); H. W. van der Marel, *Ing. Nederland.-Indië* **8**, No. 6, VII, 66-7 (1941).

tion. This oxidation may also take place at room temperature if the solution is allowed to stand for a latent period. The addition of a ml. of 10 per cent urea solution has also been advocated.[57] Platinum foil will substitute for silver as a catalyst,[58] and introduces no appreciable quantity of foreign matter into the solution. It cannot be used in silicate analysis since the formation of pertitanic acid is also catalyzed. Chloride ion interferes when either the silver or platinum catalysts are used.

The presence of 2 per cent of sulfuric acid prevents interference by small amounts of titanium.[59] Larger amounts of titanium oxide prevent the development of color in the presence of small quantities of manganese, unless excessive amounts of reagents are used; then the method is inaccurate. Where large amounts of titanium are present, it is advisable to use potassium periodate or sodium bismuthate as the oxidizing agent.[60] When the color comparison is made, the solutions should not contain more than 20 ppm. of manganese; otherwise the color is too deep for accurate reading. The errors reported vary from 1 to 7 per cent, the latter for exceedingly small amounts of manganese. The usual estimate is 2-3 per cent.

If the amount of manganese is very small, add to the developed sample containing the manganese as permanganate, developed by persulfate, 5 ml. of 1 per cent potassium iodide solution and 5 ml. of 1 per cent starch solution. After 15 minutes read the transmittance.[61]

The periodate oxidation of manganese [62] is widely applicable because it is shorter than the persulfate method, since the removal of chlorides by evaporation is unnecessary. An excess of periodate does not materially alter the color of the solution. The stability of the permanganate absorption band does not shift appreciably with changes in concentration of cation,[63] indicating that sulfuric, nitric or phosphoric acid may be used in a wide range of concentrations.[64] If the amount of manganese is very small, it is desirable that the acidity of sample and standard be similar. The color is very stable in 5-6 per cent sulfuric acid.[65] The reaction proceeds rapidly in hot solution. Spectrophotometric studies of

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[57] John H. High, *Analyst* 70, 18-19 (1945).

[58] Oskar Hackl, *Z. anal. Chem.* 112, 174-9 (1938).

[59] Oskar Hackl, *ibid.* 105, 182-99 (1936); *ibid.* 110, 401-6 (1937).

[60] George J. Hough, *Ind. Eng. Chem., Anal. Ed.* 7, 408-9 (1935).

[61] W. Teichert, *Iva* 17, 135-52 (1946).

[62] H. H. Willard and L. H Greathouse, *J. Am. Chem. Soc.* 39, 2366-77 (1917).

[63] W. M. Murray, Jr. and S. E. Q. Ashley, *Ind. Eng. Chem., Anal. Ed.* 10, 1-5 (1938).

[64] J. P. Mehlig, *ibid.* 11, 274-7 (1939).

[65] M. B. Richards, *Analyst* 55, 554-60 (1930).

the periodate method indicate that there is no fading of the end point for two months.[66] The pH probably must be below 2 for maximum color development.

With periodate, the effect of iron is negligible in the presence of a large amount of orthophosphoric acid. This also prevents precipitation of manganese iodates or periodates. If nitric acid is used, the iron content must be low or ferric periodate will precipitate. For low concentrations of manganese the color development is incomplete if the acidity is greater than 5.5 *N*. Orthophosphoric acid is preferable when large amounts of chlorides are present. In cases where the chloride concentration is extremely high, the manganese is extracted at a pH of 9 with 8-hydroxyquinoline, and the oxine oxidized by means of hydrogen peroxide and perchloric acid. The solution is then partially evaporated, potassium periodate is added, and the permanganate ion estimated.[67] Periodate causes some oxidation of chromium, this being reduced in amount by increase of acidity. Bismuth and tin precipitate, even in highly acid solutions, but silver, mercury, and lead do not if the solution is sufficiently acid.

To allow optical separation of color produced by ions of other metals, a bleached blank is prepared by adding a few drops of 0.01 per cent hydrogen peroxide to the permanganate after measurement. This permits photometric measurement of the background color. The bleaching agent is not present in sufficient concentrations to react with periodate, nor does it oxidize any other ions to colored metal peroxides. These results are more accurate and consistent than those obtained using a water blank. If the interfering ions are present in hundredfold excess, sodium azide may replace hydrogen peroxide.[68] A drop of 2 per cent sodium nitrite solution is an alternative. Conformity to Beer's law is evident at least up to 150 mg. of manganese per liter. By this method 0.2 ppm. of manganese can be determined. The minimum quantity[69] that may be determined is 0.0007 mg. and the error 0.0001 mg. for 0.0007-0.0012 mg.

The use of paraperiodate in the analysis of small quantities of manganese in sea water permits detection of 0.001 ppm. of manganese.[70] In

[66] J. P. Mehlig, *Ind. Eng. Chem., Anal. Ed.* **13**, 819 (1941); cf. *ibid.* **11**, 274-7 (1939).

[67] Dwight Williams and R. V. Andes, *ibid.* **17**, 28-31 (1945).

[68] George P. Rowland, Jr., *ibid.* **11**, 442-5 (1939).

[69] A. Broek and L. K. Wolff, *Acta Brevia Neerland. Physiol., Pharmacol., Microbiol.* **5**, 80-1 (1935).

[70] Thomas G. Thompson and Thomas L. Wilson, *J. Am. Chem. Soc.* **57**, 233-6 (1935).

the absence of reducing agents, the sample protected from evaporation retains its color indefinitely.

Procedure. *Periodate Method.* The sample may contain substantial amounts of mineral acids, particularly if it is a metallurgical sample. If chlorides are present, acidify with sulfuric acid and evaporate to sulfur trioxide fumes. Measure out a suitable aliquot, such as 25 ml., to contain 0.5-2 mg. of manganese. Adjust it so that the acid present corresponds approximately to the addition of 20 ml. of concentrated nitric acid, 2 ml. of concentrated sulfuric acid, and 5 ml. of 85 per cent orthophosphoric acid. Add a few silicon carbide chips to prevent bumping and heat almost to boiling. Carefully add 0.5 gram of potassium or sodium periodate and boil for 3 minutes. If the double salt, $Na_3H_2IO_6$, is used add 50 per cent excess. Digest just below boiling for 15 minutes to develop the color fully. If tin is being retained in solution by the presence of fluorides, avoid loss of fluoride by volatilization, otherwise precipitation will occur. Cool and dilute to 100 ml. If the distilled water to be used for dilution contains a trace of reducing agent, add 1 per cent of sulfuric acid and a crystal of periodate and boil before use. Compare with similarly prepared standards or read the color photometrically at 520 or 550 mμ and compare with a calibration curve. If chromate is present read at 575 mμ to avoid interference.

Persulfate Method. To the sample in acid solution free of chlorides, add 1 ml. of 2 per cent silver nitrate solution and 1 gram of moist potassium or ammonium persulfate per mg. of manganese. The persulfate should have been moistened a day or two before use since persulfate oxidation does not proceed smoothly when the added salt is dry, probably because of the small solubility of the salt. Heat in a water bath until the permanganate color is developed, usually about 10 minutes. Cool, dilute to the required volume, and measure colorimetrically against standards or photometrically at 525 mμ.

Bismuthate Method. To an aliquot of sample in acid solution add 0.1 gram of sodium bismuthate per mg. of manganese. Warm with continuous stirring to 50° Allow to cool and settle, and filter through a double filter into a 200-ml. flask. Wash the filter with small portions of water, receiving the washings in the flask. Make up to volume and compare with standards or read photometrically at 525 mμ.

Lead Peroxide Method. To 10 ml. of sample solution add 3 ml. of 1:1 nitric acid and place a funnel in the neck. Heat on a calcium chloride

bath boiling at 115°. When the solution begins to boil, add 0.5 gram of fine lead peroxide. The lead peroxide must show no color on heating 5 minutes with 1:1 nitric acid. It may be prepared by boiling red lead with 1:3 nitric acid, decanting the upper layer of lead nitrate solution, filtering, and washing with hot water. Boil gently for 5 minutes and remove. Let cool and settle in a dark place. Either read a portion of the clear upper layer photometrically or compare with a standard directly by any of the conventional methods.

Manganese by Formaldoxime

When an alkaline solution of a manganese salt is treated with formaldoxime, Denigès reagent,[71] a wine-red coloration instantly develops which is suitable for colorimetric estimation.[72] Neither time nor oxidation by air has any appreciable effect on the color. Iron shows a similar reaction, the color developing by air oxidation. This effect of iron can be avoided by precipitation of the iron as the orthophosphate under conditions which permit keeping manganese phosphate in solution. Alternatively,[73] precipitate the iron with zinc oxide and determine manganese in the filtrate.

By neutralizing an aliquot sample with ammonium hydroxide and treating with formaldoxime reagent, manganese can be determined in the presence of over 99.5 per cent of cobalt.[74] The presence of copper, nickel, and chromium does not interfere.[75] Calcium or magnesium with phosphate tend to interfere because of precipitation, but the addition of ammonium sulfate will increase the solubility of the phosphates and avoid this interference. Comparison must be made with a standard of very similar concentration.

Procedure. If the sample solution does not already contain phosphate, add 1 ml. of 1:100 orthophosphoric acid per 100 ml. of sample. Titrate a 5-ml aliquot of sample, containing 2 drops of methyl red indicator, with 4 per cent sodium hydroxide solution. Take the titration reading and discard the aliquot. Place a 10-ml aliquot in a 50-ml. centrifuge tube. Add from a burette an amount of 4 per cent sodium hydroxide calculated to neutralize the acidity of the sample, according to the previous titration figure Ferric and manganese orthophosphates

[71] Georges Denigès, *Compt. rend.* **194**, 895-7 (1932).
[72] C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* 9, 445 6 (1937); *ibid* 12, 307 (1940).
[73] L. C. E. Kniphorst, *Chem. Weekblad* 42, 311 16 (1946).
[74] Louis Waldbauer and Nell M Ward, *Ind. Eng. Chem., Anal Ed.* 14, 727-8 (1942)
[75] V. M. Peshkova and A. A. Ovsyannikova, *Zavodskaya Lab.* 6, 800 3 (1937).

The color developed is the same as for free chlorine, and the same standards are used. Manganese in water is an example. Other oxidizing agents give the same reaction. Neutralize a suitable sample with 10 per cent sodium hydroxide solution until just alkaline to phenolphthalein. Bubble oxygen through the solution for 10 minutes, or clean air for 30 minutes, to make sure the manganese is oxidized. If more than 0.5 ppm. of iron is present, add 5 ml. of 85 per cent orthophosphoric acid.

As reagent grind 1 gram of pure *o*-tolidine with 5 ml. of 1:5 hydrochloric acid to a thin paste. Then dissolve in water and dilute to 500 ml. Make up to 1 liter with 1:5 hydrochloric acid. Transfer the entire sample to a Nessler tube. Treat with 5 ml. of the *o*-tolidine reagent. Compare after 15-20 minutes with the permanent standards for chlorine (page 709) and multiply the result in terms of chlorine by 1.25 to give the value as manganese. Manganese standards may also be used.

Manganese in lactic acid solution gives a characteristic color change with leuco-malachite green.[82] The reagent contains 1 mg. of the dye per 100 ml. of 25 per cent lactic acid. In a typical procedure the sample is strongly acidified with 25 per cent lactic acid, about 5 per cent by volume of reagent added, the mixture allowed to stand for 10 minutes and read photometrically at around 610 mμ.

An alcoholic solution of tetramethyldiaminodiphenylmethane in the presence of glacial acetic acid, when added to manganic chloride, yields a deep blue to violet color.[83] A similar reaction is obtained with tetramethyldiphenylmethane.[84] Place 1 ml. of sample in acid solution, and 1 ml. of standard manganese solution, in 10 ml. flasks. Dilute with distilled water to about 6 ml. Add 1 ml. of a 0.5 per cent solution of tetramethyldiaminodiphenylmethane in ethanol and 1 ml. of glacial acetic acid. Mix and add 0.5 ml. of 4 per cent sodium hydroxide solution. Mix and add glacial acetic acid, drop by drop, until the maximum color is developed. Dilute to 10 ml. and compare.

Dimethyl-*p*-phenylenediamine hydrochloride is oxidized by manganese dioxide and thus permits a determination of manganese, particularly in water.[85] Add 3 ml. of 1:5 hydrochloric acid to 100 ml. of water and aerate to remove carbon dioxide. Then add 4 per cent sodium hydroxide to approximately pH 8.5. Again pass air through the solution to oxi-

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[82] G. Schwarz, O. Fischer and B. Hageman, *Deut. Molkerei-Ztg.* 64, 143-4 (1943).

[83] Ralph G. Harry, *Chem. and Ind.* 50, 796 (1931); *J. Soc. Chem. Ind.* 50, 434-6T (1931)

[84] M. A. Trillat, *Compt. rend.* 136, 1205-7 (1903).

[85] R. Schmidt, *Chem.-Ztg.* 51, 1015-16 (1927); V. Krasnova, *J. Gen. Chem.* (U.S.S.R.) 7, 1417-18 (1937).

dize the manganese. Add 2 drops of a 2 per cent aqueous solution of the reagent, followed by dropwise addition of 10 per cent citric acid solution until the color appears, and about 3 drops excess. The citric acid prevents interference by iron. If nitrite interferes, add 2 drops of 5 per cent sodium azide solution before aerating. More than 50 mg. of magnesium per liter will interfere. The test will show 0.04 mg. of manganese per liter.

Base Exchange Capacity by Manganese

The base-exchange capacity of a natural or artificial mineral such as a zeolite may be determined colorimetrically. For the purpose, the mineral is saturated with bivalent manganese which is subsequently displaced, and determined as permanganic acid.[86] This permits micro determination. The operations are long and laborious if done by hand but may be expedited by use of a shaking machine.[87] Results generally agree with those by the calcium saturation method and are high compared with results by ammonia saturation on clay-type minerals.

Procedure. *Place a 1-gram sample* of the *mineral* in a *100-ml.* centrifuge tube. Add 50 ml. of a 20 per cent solution of manganous chloride tetrahydrate solution and shake vigorously for 5 minutes. Remove the stopper and wash into the tube with 95 per cent ethanol or methanol. Similarly wash down the sides of the tube. Centrifuge for 5 minutes at 1500 rpm. and pour off the supernatant layer. Repeat this treatment 5 times. Wash in the same way with 50-ml. portions of 95 per cent ethanol or methanol until the washings no longer give a positive chloride test, usually 4 times

Recover the displaceable manganese which will not wash out with an alcohol by washing in the same way with 7.7 per cent ammonium acetate solution, repeating this process 5 times. Dilute the combined washings to 500 ml. and take an aliquot containing 0.25-0.75 mg. of manganese as sample. Evaporate to dryness and wash down the sides of the beaker with 2-3 ml. of concentrated nitric acid. Again evaporate to dryness to destroy carbonaceous matter. Repeat as necessary to complete this operation; usually twice will suffice Add acid for the determination of manganese by the periodate method (page 394) and complete.

[86] C. A. Bower and Emil Truog, *Ind. Eng. Chem., Anal. Ed.* 12, 411-13 (1940).

[87] E. Truog, J R. Taylor, Jr., R. W. Pearson, M. E. Weeks and R. W. Simonson, *Soil Sci Soc Am, Proc.* 1, 101 12 (1936).

To either of the above solutions add 3 ml. of 70 per cent perchloric acid and 1 drop of 48 per cent hydrofluoric acid. Evaporate on a hot plate until perchloric acid vapors condense in the neck of the flask. At this stage chromium will be completely oxidized.

Generate hydrogen chloride by dropping hydrochloric acid from a separatory funnel into a suction flask containing concentrated sulfuric acid, or use a cylinder of the anhydrous gas. Pass dry hydrogen chloride gas through the solution on a hot plate to volatilize chromium as CrO_2Cl_2 and tin as $SnCl_4$. Finally dilute the sample solution with 10 ml. of water for use in determination by dithizone.

Zinc-aluminum-copper and Zinc-aluminum-iron Alloys. The preparation of samples of these alloys is given under copper (page 81). Use an aliquot of the sample so prepared for determination of zinc by the dithizone method.

Silicate Rock. The zinc is isolated with lead in 1:500 hydrochloric acid under lead (page 11). Use an aliquot of the sample for determination by the dithizone method.

Soils.[5] Fuse a 5-gram sample in a platinum crucible with 8 grams of potassium pyrosulfate and add 8 grams more of pyrosulfate in small portions to prevent boiling over. Cool slowly so that the fusion will separate more readily from the crucible. Dissolve the melt in 250 ml. of water and 10 ml. of concentrated hydrochloric acid. If the calcium content is high, stronger heat than usual may be necessary to decompose the fusion. Evaporate the solution to 100 ml. on a hot plate. Filter through a Büchner funnel to remove silica and calcium sulfate. Wash the precipitate well with cold water. Evaporate the combined washings and filtrate to 100-150 ml.

The acidity is now to be adjusted to 0.4 N by addition of sodium hydroxide solution. To do this, titrate 1 ml. of the prepared sample with 20 per cent sodium hydroxide solution, using 1 ml. of bromophenol blue indicator. Use for end-point comparison, the color produced by the same amount of indicator with 1 ml. of pH 3.2 buffer solution (Vol. 1, p. 173). This result multiplied by the number of ml. of sample to be used will give the amount of 20 per cent sodium hydroxide solution required to bring the sample to the proper acidity. Add the alkali, cool the solution, and saturate with hydrogen sulfide for 15 minutes.

[5] Hugh M. Boggs and A. O. Alben, *ibid.* 8, 97-9 (1936).

Filter and wash the precipitate with 1:30 hydrochloric acid saturated with hydrogen sulfide. Make up the filtrate to a known volume.

As the necessary buffer, dissolve 65 grams of ammonium sulfate, 50 grams of citric acid, 20 ml. of 90 per cent formic acid, and 50 ml. of ammonium hydroxide in water and make up to 250 ml. Add 2.5 ml. of this to a 25-ml. aliquot of the filtrate of sample.

Prepare a special bromothymol blue indicator by dissolving 0.1 gram of the indicator in 28.5 ml. of 4 per cent sodium hydroxide solution and diluting to 250 ml. Add 0.25 ml. of this indicator to the sample and titrate to a gray color or slightly beyond with 20 per cent sodium hydroxide solution. Add 1 ml. of a 5 per cent talc suspension and saturate with hydrogen sulfide for 30 minutes.

Filter and wash the precipitate 4 times with hydrogen sulfide water containing 4 ml. of 90 per cent formic acid per liter. Wash the filter with distilled water. Establish the absence of iron in the precipitate by adding a drop of 2 per cent ammonium thiocyanate solution.[6] Dissolve the precipitate by pouring 30 ml. of 1:12 hydrochloric acid through the filter, using a 100-ml. volumetric flask as a receiver. Make up to volume by washing the filter paper repeatedly with distilled water. This preparation of sample is designed for ferrocyanide determination but by boiling off the hydrogen sulfide is suitable for other methods.

Alternatively,[7] extract 10 grams of soil sample with 40 ml. of 1:20 nitric acid and filter. Discard the first few ml. of filtrate and then collect 20 ml. Add a few ml. of concentrated sulfuric acid to this, evaporate to dryness, and ignite below 500°. Extract this residue with 5 ml. of hot 10 per cent ammonium acetate solution. Repeat the extraction and then extract with hot water. Combine the extracts and dilute to 100 ml. for the use of aliquots. Use the dithizone method.[8]

Water and Salt Solutions.[9] A sample has had lead (page 16) and copper (page 91) removed successively to give a solution containing iron and zinc. Boil this acid solution to remove hydrogen sulfide. Cool and neutralize with 1:1 ammonium hydroxide. Add 10 ml. of 10 per cent citric acid monohydrate solution. Heat to boiling and, if no calcium citrate separates, add small quantities of powdered calcium carbonate until a precipitate of about 1 gram of calcium citrate is formed. Pass hydrogen sulfide through the solution until it is cool. Let this stand for

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[6] P. V. Zimakov, *J. Phusol.* (U.S.S.R.) 24, 992-5 (1938).

[7] Hans Westerhoff, *Bodenkunde u. Pflanzenernähr.* 7, 378-84 (1938).

[8] O. Brandlie and H. Bergin, *Tids. Kjemi, Bergvesen Met.* 2, 88-9 (1942).

[9] Cf. Noel L. Allport and C. D. B. Moon, *Analyst* 64, 395-402 (1939).

several hours, part of the time on a steam bath, until the supernatant liquid is clear. The precipitate contains the zinc.

Filter and wash the paper with 2 per cent ammonium thiocyanate solution. Dissolve the precipitate from the filter with hot 1:9 hydrochloric acid. If the filtrate is reddish in color, reprecipitate the zinc as before. Dispel any turbidity of the filtrate due to colloidal sulfur by boiling. When the filtrate is clear and colorless, dilute to a known volume and take an aliquot for determination by the ferrocyanide method.

A sample may also be prepared for determination by the dithizone method.[10] A sample may be used as received, or diluted, or concentrated to 50 ml. For removal of copper contamination, if present, acidify and extract the copper with dithizone (page 407). Then neutralize the added acid. Add 5 ml. of 10 per cent ammonium citrate solution. Add 1:1 ammonium hydroxide until faintly basic. Shake with 5 ml. portions of 0.01 per cent dithizone in carbon tetrachloride. Withdraw the extract and shake with successive portions until one remains green. Combine the extracts which now contain the zinc and discard the aqueous layer. Wash the combined extracts with 2 ml. of water and discard the wash liquid. Extract the carbon tetrachloride solution with 10 ml. of 0.02 N hydrochloric acid to remove the zinc. Extract the solvent layer a second time and combine the extracts. Dilute the extract to a known volume and use as sample by the mixed color method. As little an amount as 0.01 mg. of zinc per liter can be isolated by this method.[11]

Biological Samples.[12] Store silica evaporating dishes in 1:1 nitric acid when not in use, to remove surface contamination. Weigh out 10-20 grams of tissue into such a dish. Add 10 ml. of redistilled concentrated nitric acid and evaporate to dryness on a hot plate. Ignite in a muffle at 500° to destroy organic matter. If necessary, let cool from time to time, add a few ml. of the redistilled nitric acid, dry, and again ignite. Take up the ash in 1:3 hydrochloric acid. Do not filter but dilute the filtrate and washings to a known volume.

Tissue.[13] Let a 25-gram sample of ground tissue stand with 50 ml. of concentrated nitric acid in a warm place for an hour. Heat on a

[10] Georg Gad and Kate Naumann, *Gas u. Wasserfach* 82, 168-9 (1939).

[11] K. Heller, G. Kuhla, F. Machek, *Mikrochemie* 18, 193-222 (1935).

[12] Jacob Cholak, Donald M. Hubbard and Roland E. Burkey, *Ind. Eng. Chem., Anal. Ed.* 15, 754-9 (1943).

[13] Alexander O. Gettler and R. Bastian, *Am. J. Clin. Path.* 17, 244-9 (1947).

steam bath until disintegration, other than of fat, is complete. Cool and filter the fat on glass wool, using a Kjeldahl flask as receiver. Add 6 ml. of concentrated sulfuric acid and a few glass beads and evaporate the nitric acid. If the residue shows signs of solidifying, add more concentrated sulfuric acid. Add a mixture of two parts of 72 per cent perchloric acid and one part of concentrated nitric acid, dropwise, until sulfur dioxide is evolved and the solution is colorless. Usually about 5 ml. will suffice. Cool and add 10 ml. of water. Boil off sulfur dioxide, then dilute to about 50 ml.

If cadmium is present, heat to 70° and pass in hydrogen sulfide until precipitation is complete, let stand for 30 minutes, and filter. Wash the precipitate well, collecting the washings with the filtrate.

Concentrate to about 10 ml., during which hydrogen sulfide must be removed if it was added. Add 2 ml. of 20 per cent sodium potassium tartrate solution and make alkaline to metacresol purple with concentrated ammonium hydroxide. Continue dropwise addition until alkaline to methyl red but acid to litmus.

Prepare a liter of buffer solution containing 225 grams of sodium thiosulfate, 5 grams of potassium cyanide, 45 grams of sodium acetate, and sufficient 1:4 acetic acid to adjust the pH to 5.5. Add 50 ml. of this buffer and dilute to about 75 ml. as a sample ready for dithizone extraction.

For the dry method,[14] thoroughly ash a suitable sample below 450°, treat with 1:3 hydrochloric acid, and allow to stand for several hours. Filter and evaporate the filtrate and washings to dryness. Add 1.2 ml. of 1:1 hydrochloric acid and bring the volume to 15 ml. Saturate the solution with hydrogen sulfide for 15-20 minutes. Allow to stand for 8 hours, filter, and wash thoroughly. Evaporate the filtrate and washings to dryness Take up the residue with 15 ml. of 1:25 acetic acid and again saturate the solution with hydrogen sulfide. Under these conditions zinc sulfide is precipitated, but iron, nickel and cobalt remain in solution. Let the precipitate settle for 12-24 hours, then add 0.25 gram of aluminum oxide or talc. Stir well and wash the precipitate with 1:25 acetic acid saturated with hydrogen sulfide until the washings give no test for iron, cobalt, or nickel. Finally discard the washings, dissolve the zinc sulfide in 2 ml. of 1:4 hydrochloric acid, filter, and dilute the filtrate and washings to a known volume for use of an aliquot by the dithizone method.

[14] P. V. Zimakov, *J. Physiol.* (U.S.S.R.) 24, 992 5 (1938).

Urine or Feces. Ash 10-100 ml. of urine or 1-10 grams of feces with 1-10 ml. of concentrated sulfuric acid and a few ml. of concentrated nitric acid. Evaporate to sulfur trioxide fumes in order to drive off all nitric acid. Dilute the cooled residue with 10 volumes of water. Add 2 drops of 10 per cent copper sulfate solution and pass in hydrogen sulfide for 20 minutes. This removes copper originally present as well as that added. Filter at once and evaporate the filtrate and washings to dryness. Heat until substantially all the excess of sulfuric acid is driven off. Dissolve the residue in water and dilute to 20 ml. Titrate 5 ml. with 0.01 *N* sodium hydroxide solution to determine the acidity. Neutralize exactly the remaining volume of sample without adding indicator, filter, and dilute to a known volume. The sample may also be ashed in a muffle[15] below 450° and the ash dissolved in acid and copper precipitated as above.

Foodstuffs.[16] The preparation of this sample provides for wet oxidation of the sample, elimination of lead, copper, cadmium, bismuth, antimony, tin, mercury, and silver as sulfides with added copper as scavenger. Cobalt and nickel are simultaneously eliminated by extracting the metal complexes of α-nitroso-β-naphthol and dimethylglyoxime with chloroform. The zinc dithizonate is then extracted with carbon tetrachloride and the zinc transferred to dilute hydrochloric acid.

Weigh a representative sample not exceeding 25 grams of material. This sample should contain 0.025-0.1 mg. of zinc. If the sample is a liquid, evaporate to a small volume. Add concentrated nitric acid and heat cautiously until the first vigorous reaction subsides somewhat. Add 2-5 ml. of concentrated sulfuric acid. Continue heating, adding more concentrated nitric acid in small increments if necessary to prevent charring, until fumes of sulfur trioxide are evolved and the solution remains clear and almost water white. Add 0.5 ml. of 72 per cent perchloric acid and continue heating until the perchloric acid has been almost completely removed. Allow to cool and dilute to about 40 ml.

The next step is a sulfide separation. Add 2 drops of methyl red indicator solution and 1 ml. of 0.8 per cent cupric sulfate solution. Add 1:1 ammonium hydroxide until the solution is neutral. Add 1 ml. of 1:1 hydrochloric acid. The pH of the solution at this point as measured with a glass electrode is 1.9-2.1. Pass a stream of hydrogen sulfide into

[15] Phebe K. Thompson, *J. Ind. Hygiene* **7**, 358-70 (1925).

[16] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, pp. 479-81. Association of Official Agricultural Chemists, Washington, D. C. (1945).

the solution until precipitation is complete. Filter through fine textured paper which has been previously fitted to the funnel and washed with 1:20 hydrochloric acid, then with redistilled water. Boil the filtrate gently until all odor of hydrogen sulfide has disappeared. Then add 5 ml. of saturated bromine water and continue boiling until excess bromine has been expelled. Allow this to cool, neutralize to phenol red with 1:1 ammonium hydroxide, and add 0.2 ml. of 1:1 hydrochloric acid. Dilute to a known volume for the use of aliquots. For optimum conditions of measurement, the solution after dilution should contain 0.0002-0.001 mg. per ml.

For the next operation a buffer is required. Dissolve 225 grams of ammonium citrate monohydrate in water and make alkaline to phenol red with concentrated ammonium hydroxide. Add 75 ml. of concentrated ammonium hydroxide in excess and dilute to 2 liters. Extract this solution immediately before use with a solution of dithizone in carbon tetrachloride until the solvent layer is clear bright green. Remove excess dithizone by repeated extraction with plain carbon tetrachloride. It is essential that excess dithizone be entirely removed, otherwise zinc will be lost during the elimination of copper and nickel.

Transfer a 20-ml. aliquot of the prepared solution to a 125-ml. separatory funnel. Add 5 ml. of the ammonium citrate buffer, 2 ml. of 0.2 per cent dimethylglyoxime solution in 1:100 ammonium hydroxide, and 10 ml. of 0.05 per cent α-nitroso-β-naphthol solution in chloroform. Shake for 2 minutes. Discard the solvent layer and extract with 10 ml. of chloroform to remove residual α-nitroso-β-naphthol. Discard this solvent layer also.

The next step is the isolation of zinc. To the aqueous phase following removal of nickel and cobalt which at this point has a pH of 8.0-8.2, add 2.0 ml. of dithizone reagent (page 415) and 10 ml. of carbon tetrachloride. Shake for 2 minutes and let the phases separate. Remove the aqueous layer as completely as possible. Wash down the sides of the separatory funnel with 25 ml. of water and, without shaking, again draw off the aqueous layer. Add 25 ml. of 0.04 *N* hydrochloric acid and shake for a minute to transfer the zinc to the acid layer. Drain off and discard the solvent, being careful to dislodge and remove the drop that usually floats on the surface. This acid solution is a prepared sample ready for dithizone determination using the bicolor method.

A technic for sulfide separation of a fraction containing copper and zinc has been given under copper (page 98). Use an aliquot for zinc.

nitric or perchloric acid is liable to result in loss of zinc, and the oxidizing agent must be completely removed.[22]

Moisten the cooled ash with a little water and add 10 ml. of 1:12 hydrochloric acid, more if necessary. Heat on a steam bath until all soluble substances have been dissolved. Add 5-10 ml. of hot water. Filter off the insoluble matter on a filter paper that has been washed with two 5-ml. portions of hot 1:12 hydrochloric acid, then with hot water until free of acid. Collect the filtrate in a 100-ml. volumetric flask. Wash the filter with hot water until the washings no longer react acid to methyl red. Add a drop of methyl red indicator to the filtrate in the 100-ml. flask, neutralize with 1:15 ammonium hydroxide, and add 4 ml. of 1:12 hydrochloric acid. Allow the contents of the flask to cool and make up to volume.

The next step is to isolate the metals which form dithizone complexes. Depending on the history of the sample this may be complex or simple, or may even be by-passed. Pipet an aliquot of the sample solution containing not more than 0.03 mg. of zinc into a 125-ml. separatory funnel. Add 1 ml. of 0.2 N hydrochloric acid for each 5 ml. of sample solution less than 10 ml. taken or 1 ml. of 0.2 N ammonium hydroxide for each 5 ml. over 10 ml. taken. In many cases the sample can be adjusted to 10 ml.

Prepare an ammonium citrate solution as follows: Dissolve 113 grams of the monohydrate in a liter of water. Add concentrated ammonium hydroxide, somewhat over 40 ml., to adjust the pH to 8.5 as measured by a glass electrode. Add 10 ml. of dithizone reagent (page 415) and extract. Repeat until the extract is a clear green. Filter the aqueous layer and store in Pyrex. To 10 ml. of this solution, add 1.4 ml. of 1:15 ammonium hydroxide and make up to 40 ml. Add to the sample, cool, and transfer to a separatory funnel with a minimum of water. The pH of the final solution should be 8.0-8.5.

Add 10 ml. of the dithizone reagent, shake for 30 seconds, and add additional 5 ml. portions of dithizone reagent until an excess is evident from the orange-yellow color in the aqueous layer. If excess dithizone is not present, add more of the reagent until after shaking an excess is indicated. Shake down the drop of extract from the surface and draw off the extract into a second separatory funnel as completely as possible without allowing any of the aqueous layer to enter the stopcock bore. Rinse down the carbon-tetrachloride extract from the surface of the aqueous layer with a 2-ml. portion of clear carbon tetrachloride.

[22] P. L. Hibbard, *ibid.* 9, 127-31 (1937).

Run this extract into the second separatory funnel without permitting the aqueous phase to enter the stopcock bore. Repeat this rinsiug process as many times as necessary to flush the extract completely into the second separatory funnel. Add 5 ml. of clear carhon tetrachloride to the first separatory funnel. Shake for 30 seconds and allow the layers to separate. The carhon tetrachloride layer at this point will have a clear green color if the metals that form dithizone complexes have heen completely removed from the aqueous phase by previous extractions.

Run off the solvent layer into the second separatory funnel and flush down the extract from the surface and out of the separatory funnel os before. If the last extract does not possess a distinct clear green color, repeat the extraction with 5-ml. portions of clear carhon tetrachloride and also the flushing-out process, until complete extraction of the dithizone complex-forming metals is assured. Discard the aqueous phase.

Next the zinc is to he separated from copper. Pipet 50 ml. of 0.02 *N* hydrochloric acid into the separatory funnel containing the solvent solution of dithizonates. Shake vigorously for 1.5 minutes and allow the layers to separate. Shake down the drop from the surface of the oqueous phose and run off as completely as possible the solvent phase that contains all the copper dithizonate, without ollowing auy of the aqueous phase, which contains all the zinc, to enter the stopcock hore. Rinse down the carhon tetrachloride extract from the surfoce of the aqueous phase and rinse out the stopcock hore with 2-ml. portions of clear solvent, as in the first extraction, until all traces of green dithizone have heen washed out of the separatory funnel. Shake down the drop of solvent from the surface of the aqueous phase and run off the solvent as completely as possible without allowing any aqueous phase to enter the stopcock bore. Remove the stopper from the separatory funnel and lay it across the neck until the small quantity of solvent on the surface of the aqueous phase has evaporated. The solution so obtained is o sample for determination with dithizone hy the mixed-color method.

Separation of Copper, Zinc, Bismuth, Lead, and Tin. Details of the separation are given under lead (page 33). The solution thus prepared contains the copper and zinc, free from the other metols.

Separation of Zinc from Nickel and Cobalt. Add 1:1 ammonium hydroxide to the sample solution to approximate neutrality. Add 5 per cent potassium cyanide solution dropwise until the precipitate is dissolved. Each ml. of 5 per cent potassium cyanide solution will fix about 7 mg. of nickel or cobalt as the complex.

hydrochloric acid to adjust the pH to 3-4, then sodium ... excess until the pH is 5-5.5. Extract the zinc with dithizone in carbon tetrachloride.

Separation of Zinc from Tin. When a sample contains tin, add 5 ml. of a 20 per cent solution of bromine in concentrated hydrobromic acid. Evaporate to dryness. This volatilizes the tin as the bromide. Add 0.5 ml. of concentrated hydrochloric acid and 1.5 ml. of concentrated nitric acid to the residue. Evaporate to dryness and take up the residue in 0.5 ml. of 1:10 nitric acid.

Removal of Iron as Fluoride. This has been outlined for separation from copper (page 107). For separation from zinc, modify in order to have 0.5-1.0 ml. of concentrated hydrochloric acid per 100 ml. of sample. Unless carried out hot, the precipitate will sorb appreciable amounts of zinc.

Standard

Place 0.25 gram of pure zinc in a 250-ml. Pyrex volumetric flask. Add 50 ml. of water and 1 ml. of concentrated sulfuric acid. Heat gently on a steam bath until the zinc dissolves. Cool, make up to volume, and store in the flask. This contains 1 mg. of zinc per ml. From this stock solution, pipet a 10-ml. aliquot and make up to 1 liter. This will contain 0.01 mg. per ml.

Zinc by Dithizone

Diphenylthiocarbazone, commonly referred to as dithizone, dissolved in carbon tetrachloride or chloroform forms a red, water-insoluble keto complex with zinc in slightly alkaline solutions. This complex is sufficiently sensitive and stable to be used as a basis for a colorimetric method. It permits the determination of 0.001-0.100 mg. of zinc with an accuracy of 5-10 per cent and sensitivity to 0.005 ppm. Several metals form colored dithizone complexes, but by controlling the pH and by the use of buffer and complex-forming agents the method is made selective.[23]

Complete extraction of zinc dithizonate is obtainable over the pH range of 5-10.[24] The critical pH of zinc dithizonate is about 10; above

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[23] For more detailed discussion of this reagent and the precautions necessary in its use refer to page 3.

[24] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* **11**, 66-72 (1939); W. Y. Gary, *J. Assoc. Official Agr. Chem.* **25**, 352-63 (1942); Kurt Buch, *Finska Kemistsamfundets Medd.* **53**, 25-37 (1944).

that the complex becomes unstable. Such a method of extraction is easiest way to isolate zinc from many varieties of samples. Many metals are extractable only from solutions of low pH. At a pH of 4.0-5.5 the thiosulfate complexes of silver, gold, mercury, copper, bismuth, lead, and cadmium are more stable than the dithizonates. Therefore, at that pH they are fixed. Zinc forms a weak complex with thiosulfate so that much thiosulfate reduces the sensitivity of the method.

The technic essentially consists of shaking the sample at a controlled pH with a dithizone-chloroform or dithizone-carbon tetrachloride solution to extract zinc as the dithizonate. Chloroform more completely removes zinc from ammoniacal solution and carbon tetrachloride is a more effective solvent for zinc in acid solution. A procedure has been devised whereby the two solvents were used, each under their most favorable pH conditions.[25] About 25 per cent greater intensity of color in chloroform is obtained. The presence of resorcinol at about pH 9 expedites extraction with dithizone in chloroform.[26] Zinc dithizonate may be determined colorimetrically as modified by excess dithizone in the bicolor method. In the monocolor method, excess dithizone is washed from the solvent with dilute ammonium hydroxide or sodium sulfide solution.

If other metals are present that do not greatly exceed the amount of zinc, determination of zinc may be made without separating these metals.[27] If copper, lead, mercury, bismuth, cobalt, tin, or nickel is present up to 10 times the amount of zinc, or cadmium to half the amount, satisfactory determinations can be made by using a solution of sodium diethyl dithiocarbamate in 0.07 per cent ammonium hydroxide to fix these metals in solution. The carbamate will fix copper in weakly alkaline solutions after other interfering metals have been extracted in acid solution.[28] Although the carbamate causes a reduction in the intensity of the dithizone color, accurate results may be obtained by keeping all conditions constant in extractions.

Interfering metals are also eliminated by adding sodium thiosulfate or potassium cyanide[29] at a pH of 4.0-4.5. Interference by calcium, iron, and phosphate is prevented by addition of a citrate buffer. Aluminum interferes if present in large amounts. If lead is present, the use

[25] Lewis G. Bricker, Sidney Weinberg and Kenneth L. Proctor, *Ind. Eng. Chem., Anal. Ed.* 17, 661-3 (1945).
[26] Noel L. Allport and C. D. B. Moon, *Analyst* 64, 395-403 (1939).
[27] K. Heller, G. Kuhla and F. Machek, *Mikrochemie* 18, 193-222 (1935).
[28] H. J. Wichmann, *Ind. Eng. Chem., Anal. Ed.* 11, 66-72 (1939).
[29] E. B. Sandell, *ibid.* 29, 464-9 (1937).

ide instead of chloroform as the solvent permits a
ation. Less than one-half as much cadmium as zinc
tion to be analyzed, otherwise a preliminary separa-
etals dissolved in the solvent phase may be trans-
is layer by adding thiosulfates or iodides in acid
agents must be absent from the final solution.
be lubricated with petrolatum.

Cobalt and nickel may be removed by extracting the cobalt as the α-nitro-β-naphthol complex, and nickel as the dimethylglyoxime complex with chloroform.[30] The presence of ammonium citrate [31] or potassium tartrate prevents the precipitation of hydroxides of aluminum and iron and phosphates of silver, barium, lead, iron, and mercury. Silver,[32] mercury,[33] and bismuth [34] are separated to remove interference. Copper [35] is often precipitated as sulfide, preliminary to the extraction of zinc from alkaline solution.

Procedure. In addition to the usual precautions, run a blank for zinc on all reagents and apparatus, which should be as zinc-free as possible. Apply this as a correction factor in the determination of the unknown. The contamination should not exceed 2.5 ppm.[36] The length of time the aqueous solutions remain in contact with the dithizone solutions and the time and vigor of mixing should be kept as constant as possible.

Bicolor Method.[37] This is preferable to the monocolor method. Copper must be no more than double the amount of the zinc. Lead has

[30] O. R. Alexander and L. V. Taylor, *J. Assoc. Official Agr. Chem.* 27, 325-31 (1944).

[31] Hans Westerhoff, *Bodenkunde u. Pflanzenernähr.* 7, 370-84 (1938).

[32] H. Fischer, O. Leopoldi and H. v. Uslar, *Z. anal. Chem.* 101, 1-23 (1935).

[33] W. O. Winkler, *J. Assoc. Official Agr. Chem.* 18, 638-44 (1935); H. Fischer and Grete Leopoldi, *Z. anal. Chem.* 103, 241-57 (1935).

[34] C. E. Willoughby, E. S. Wilkins, Jr. and E. O. Kraemer, *Ind. Eng. Chem., Anal. Ed.* 7, 285-6 (1935).

[35] W. Deckert, *Z. anal. Chem* 100, 386-90 (1935).

[36] E. B. Holland and W. S. Ritchie, *J. Assoc. Official Agr. Chem.* 22, 333-8 (1939); *ibid.* 25, 393-4 (1942).

[37] E. B. Holland and W. S. Ritchie, *J. Assoc. Official Agr. Chem.* 22, 333-8 (1939); Hale Cowling and E. J. Miller, *Ind. Eng. Chem., Anal. Ed.* 13, 145-9 (1941); O. Kortüm and B. Finckh, *Die Chemie* 57, 73-4 (1944); Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, pp. 123-6. Association of Official Agricultural Chemists, Washington, D. C. (1945).

little effect, nickel somewhat more. As a sample, take an aliquot and taining not more than 0.03 mg. of zinc. Adjust the volume to approximately 50 ml. and the acidity to approximately 0.02 *N*. A convenient method of adjustment of acidity is to neutralize and then to add 0.15 ml. of 1:1 hydrochloric acid.

To prepare the reagent, dissolve 0.20 gram of dithizone in 500 ml. of carbon tetrachloride which has been distilled over anhydrous sodium sulfate. Filter to remove any insoluble matter and add 2 liters of 0.02 *N* ammonium hydroxide, prepared by diluting 40 ml. of *N* ammonium hydroxide to 2 liters. Shake to extract the dithizone into the aqueous phase, separate, and discard the solvent fraction. Extract the aqueous layer with 100-ml. portions of carbon tetrachloride until the extract is clear green. Discard these carbon tetrachloride extracts which contain the impurities. As the next step, transfer the purified dithizone from the aqueous phase to carbon tetrachloride. For this add 45 ml. of 1:12 hydrochloric acid to the aqueous ammoniacal solution. Then add 500 ml. of carbon tetrachloride and shake vigorously to extract. Let separate and discard the aqueous phase. Dilute the nonaqueous layer to 2 liters with carbon tetrachloride. Store in a brown bottle in a dark, cool place.

As a buffer take 100 ml. of extracted ammonium citrate described for preparation of samples of plant tissue (page 410). Add 30 ml. of concentrated ammonium hydroxide and just before use mix 9 volumes of this buffer with 1 volume of fresh 0.25 per cent aqueous diethyl dithiocarbamate.

Add 50 ml. of this reagent and 10 ml. of the dithizone reagent to the sample in 0.02 *N* hydrochloric acid. Shake for 1 minute and allow the phases to separate. Flush out the stopcock and stem of the separatory funnel with about 1 ml. of the extract and collect the balance in a test tube. Pipet 5 ml. of the extract into a 25-ml. volumetric flask, dilute to the mark with clear carbon tetrachloride and read the transmittance of this diluted solution with a photoelectric colorimeter. Use a Sextant Green (Corning No. 401) filter, or the equivalent. Protect this final extract from sunlight as much as possible and read within 2 hours. Maximum light absorption occurs at 530 mμ[38] although readings have been taken as low as 520 mμ to decrease dithizone interference. Excess dithizone may also be read with a red filter as an indirect determination.

Monocolor Method. Lead and copper must be absent. Transfer an aliquot of sample containing 0.005-0.01 mg. of zinc to a separatory fun-

[38] J. Schwaibold, *Biochem. Z.* 297, 324-31 (1938).

e buffer by dissolving 400 grams of citric acid in
t 1:1 ammonium hydroxide to make the solution
ol blue and make up to 1 liter. Before use, dilute a
ual amount of water and shake with di-*beta*-
e-chloroform solution until the latter regains its
0 ml. of this 20 per cent ammonium citrate solution
y funnel.

Transfer an aliquot of sample solution containing under 0.50 mg. of zinc to the separatory funnel. To the contents of the separatory funnel, add 4 drops of a 0.1 per cent thymol blue solution and 1:1 zinc-free ammonium hydroxide to a pH of 9.5. Dissolve 1.25 grams of sodium diethyldithiocarbamate in 100 ml. of water and add 4 ml. of this solution to the sample. Make up with water to 100 ml. Shake for 1 minute with 5 ml. of the reagent of lower concentration to determine the concentration of reagent to use.

A faint violet indicates less than 0.005 mg. of zinc, whereas a deeper red indicates more than 0.005 mg. In the latter case add 5 ml. of the stronger reagent solution and shake for 1 minute. If necessary, repeat extractions with 5-ml. portions of the selected solution until the last portion in the agitated solution retains its original blue-green color, draining each portion into a second funnel before adding the next. Wash the combined chloroform extracts with 50 ml. of water and transfer to a third funnel.

Remove di-*beta*-naphthylthiocarbazone in the aqueous phase by shaking twice with 5-ml. portions of chloroform and add to the combined chloroform fractions. Dilute to a known volume and shake an aliquot containing not more than 0.05 mg. of zinc with 50 ml. of zinc-free 1:60 hydrochloric acid. Discard the chloroform phase. Remove entrained di-*beta*-naphthylthiocarbazone from the hydrochloric acid phase, which contains the zinc, by washing twice with 5-ml. portions of chloroform. The acid contains all the zinc free from copper, nickel, cobalt, iron, mercury, silver, phosphates, aluminum, sulfates, and most of the bismuth.

Transfer 45 ml. of 1:40 ammonium hydroxide to a separatory funnel. Add 1 ml. of carbamate solution and 5 ml. of the more dilute reagent solution and shake. Discard the extract and wash the ammoniacal solution with 5 ml. of chloroform, which is also discarded. This removes any zinc in the reagent so prepared. Allow any chloroform on the surface to evaporate and add the ammonia-carbamate solution to the hydrochloric acid extract. Mix and shake for 1 minute with 10 ml. of the reagent solution of the proper range. If less than 0.005 mg. of zinc is present, pipet

a 5-ml. aliquot of the extract into a 25-ml. glass-stoppered cylinder and make up to volume with chloroform. Fill a 2.5-cm. cell and obtain the reading at 550 or 650 mμ. If the higher range is used, pipet 5 ml. of the extract into a 100-ml. cylinder, make up to volume with chloroform, and read in a 1-cm. cell. Determine the amount of zinc from standard curves obtained from following the same procedure with known amounts of zinc.

Zinc by 5-Nitroquinaldic Acid

5-Nitroquinaldic acid completely precipitates zinc within 30 minutes in solutions ranging in pH from 2.5 to 8. Reduction with stannous chloride yields a deep orange solution that may be measured colorimetrically[44] for determination of 0.05-1.0 mg. The absorption band is from 400 to 600 mμ.

The reagent is prepared from 5-nitroquinaldic acid, crystallized from water with two molecules of water of hydration. One ml. of the prepared solution is equivalent to approximately 1 mg. of zinc. Other metals including silver, lead, mercury, copper, iron, nickel, manganese, and cobalt also form precipitates in weakly acid solutions, and should be removed by the hydrogen sulfide method (page 402).

Ammonium chloride or sodium chloride formed by the addition of acid to the alkali solution must not exceed 0.025 gram per ml., otherwise the zinc will not be completely precipitated. High concentrations of acid reduce the depth of color; however, the color is not sensitive to slight changes in stannous chloride concentration. Higher temperatures intensify color, hence comparisons with the standards must be made at the same temperature. The precipitate does not sorb excess reagent when a substantial excess is present. The color of the solution is stable for at least 24 hours.

Procedure. Measure an aliquot of solution from which interfering elements have been removed with hydrogen sulfide, to contain from 0.05-1.00 mg. of zinc, into a 30-ml. beaker. Bring the volume to 5-10 ml. by evaporating or diluting. Add a drop of 0.1 per cent solution of methyl red in 95 per cent ethanol. Make the solution just alkaline with 1:4 ammonium hydroxide. Add 1-2 drops of 50 per cent acetic acid to make

[44] Priyadaranjan Rây and Mukul Kumar Bose, *Z. anal. Chem.* 95, 400-14 (1933); Priyadaranjan Rây and Anil Kumar Majumdar, *ibid.* 100, 324-7 (1935); Priyadaranjan Rây and Mukul Kumar Bose, *Mikrochemie* 17, 11-13 (1935); 18, 89-91 (1935); W. L. Lott, *Ind. Eng. Chem., Anal. Ed.* 10, 335-8 (1938).

to that for aluminum (page 258). Insofar as zinc ammonium phosphate can be isolated[50] free from other phosphates, it can be dissolved and the phosphate determined as an indirect method. The fluorescence of a turbidity of zinc 8-hydroxyquinolinate has been used as a quantitative method, accurate to 0.02 mg. over the range 0.05-0.60 mg.[51] Other ions precipitated by 8-hydroxyquinoline interfere.

Zinc may be estimated turbidimetrically[52] or nephelometrically[53] as the white, flocculent sulfide, if present in a concentration of 0.05 per cent or less. If less than 0.5 mg. of zinc per liter is present, the accuracy of estimation is to about 0.1 mg.

If urobilin or stercobilin is added to a solution containing zinc, a pink color results, which, when made faintly alkaline, changes to yellow with green fluorescence. This can be compared with a series of standards between 10-30 minutes after preparation, using a strong arc light and an ultraviolet filter. This method can be used within the range of 0.001-0.01 mg. with an accuracy of 10 per cent.[54] It will detect 0.001 mg. of zinc. Many metals interfere.

Resorcinol in alkaline solution produces a blue color with zinc.[55] The character of this alters on exposure to the air. The addition of hydrochloric acid turns the solution red and this color is not altered by exposure. In the absence of air the blue compound is not formed in alkaline solutions containing ammonium hydroxide.[56] In the absence of ammonium hydroxide a green color is developed at pH 9.2 and a red one on the acid side. Addition of ammonium hydroxide changes this irreversibly into the blue and red compounds so that over the range pH 4.3-5.9 the color is changing and is violet. Both colors can be extracted with amyl alcohol. Doubt as to the reliability of the method in alkaline solution is raised by separation of crystals of resorcinol from anhydrous ether which give the same blue on exposure to air. Zinc catalyzes the development of this blue color.[57] Calcium, nickel, and cobalt must be absent. A sample solution suitable for examination by this method must contain between 0.0001 mg. and 0.2 mg. of zinc per ml.

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[50] W. R. Todd and C. A. Elvehjem, *J. Biol. Chem.* 96, 609-18 (1932); P. L. Hibbard, *Ind. Eng. Chem., Anal. Ed.* 6, 423-5 (1934).

[51] Lynne L. Merritt, Jr., *ibid.* 16, 758-60 (1944).

[52] L. W. Winkler, *Z. angew. Chem.* 26, 38 (1913).

[53] Ludwig Pincussen and Ernst Brück, *Biochem. Z.* 265, 58-60 (1933).

[54] Robert E. Lutz, *J. Ind. Hygiene* 7, 273-89 (1925).

[55] Angel del Campoy Cerdan and J. de la Puente, *Anales soc. espan. fis. quim.* 11, 98-108 (1913).

[56] Ligor Rey and M. Faillebin, *Compt. rend.* 188, 1679-88 (1929).

[57] Herm. Mohler and Rose Widmer, *Mitt. Lebensm. Hyg.* 22, 130-3 (1931).

CHAPTER 22

TITANIUM

Although it is an uncommon element, titanium is not a rare element because it is widely distributed in nature. Titanates occur as salts of heavy metals. Titanium alloys are also frequently encountered. Samples will usually be inorganic, but its use as a pigment contradicts that generalization.

Oxidation of sulfate solutions with hydrogen peroxide is the most common method of estimating titanium colorimetrically, although several colored organic compounds have also been used in recent years.

Samples

Aluminum and Aluminum Alloys.[1] Prepare an acid mixture of 120 ml. of 1:3 sulfuric acid, 60 ml. of concentrated hydrochloric acid and 20 ml. of concentrated nitric acid. Dissolve 1 gram of sample in 30 ml. of this mixture and evaporate until copious fumes of sulfur trioxide are given off for 5 minutes. Cool and take up the residue in 10 ml. of 1:3 sulfuric acid and 100 ml. of water. Boil until all sulfates are dissolved and filter with the assistance of paper pulp. Wash the residue with hot water until sulfate-free and save this filtrate and washings. Dry the paper and ignite in platinum. Fuse the residue with about 2 grams of sodium carbonate and let cool. Dissolve the residue in 1:3 sulfuric acid and transfer to a casserole with sufficient sulfuric acid so that on fuming it will not solidify. Set aside the crucible after washing. Evaporate the sulfuric acid solution to strong fumes, cool, and dilute to about 100 ml. Warm until salts are dissolved and filter. Wash well and set aside this filtrate and washings.

Dry the residue and ignite in the previously used platinum crucible at 500°. Finally ignite at 1000°. Moisten the ash with water, add a few ml. of 1:3 sulfuric acid, and an equal volume of 48 per cent hydrofluoric acid. Evaporate to dryness and ignite. Fuse the residue with a small amount of potassium bisulfate, cool, and take up in 1:20 sulfuric acid. Combine this with the earlier filtrate and washings. Evaporate to about 100 ml. and add 10 ml. of 1:1 sulfuric acid. Add 3 grams of iron-free

[1] "ASTM Methods of Chemical Analysis of Metals," p. 134, American Society for Testing Materials, Philadelphia, Pa. (1943).

and filter. Wash the residue with hot water and discard the filtrate and washings. Ignite the residue in platinum and fuse the ash with 2 grams of sodium carbonate and 0.1 gram of sodium nitrate. Decompose the cooled melt by heating with water on a hot plate. Filter and wash with hot water. Discard these washings.

Treat the paper and residue with hot 1:10 sulfuric acid. Dilute this solution to a known volume with the same acid and take an aliquot as sample. If the final solution shows any color of iron, add a drop or two of 85 per cent orthophosphoric acid. Develop the color by hydrogen peroxide.

Uncombined Titanium in Steel. Add 5 grams of sample to 100 ml. of 1:9 hydrochloric acid and heat. When reaction ceases, filter and wash the filter thoroughly with hot 1:100 hydrochloric acid until iron is completely removed. Reserve the filter for combined titanium. Dilute the filtrate to 300 ml. and proceed as for total titanium starting at "Then add 1:1 ammonium hydroxide until faintly alkaline."

Combined Titanium in Steel. The filter containing this was reserved in determination of uncombined titanium. Ignite at a low temperature until ashing is complete. Fuse with about 3 grams of sodium carbonate and 0.1 gram of potassium nitrate. Take up the melt in hot water and filter. Wash the filter with hot water and discard the filtrate and washings. Ignite the residue and paper. Fuse the ash with 3 grams of potassium bisulfate to a clear melt. Take up the residue in 1:9 sulfuric acid and filter if necessary. Dilute to a known volume and use an aliquot for development of titanium by hydrogen peroxide.

Titanium Steel.[6] Heat 0.5-1.0 gram of sample gently with 100 ml. of 1:4 hydrochloric acid until reaction is complete. If the copper content of the sample is inappreciable go directly to the cupferron precipitation. Otherwise add 10 ml. of concentrated sulfuric acid and heat to sulfur trioxide fumes. Let cool and dilute to about 100 ml. Filter the solution through a small paper containing paper pulp and wash thoroughly with hot 1:9 sulfuric acid. Reserve this solution for cupferron precipitation. Treat the paper with the acid-insoluble matter in a beaker with 25 ml. of 3:7 nitric acid and heat until the copper has dissolved. Add 50 ml. of water and make sufficiently alkaline with concentrated ammo-

[6] "ASTM Methods of Chemical Analysis of Metals," p. 74, American Society for Testing Materials, Philadelphia, Pa. (1943).

nium hydroxide to redissolve copper hydroxide. Heat to boiling, filter, and wash the precipitate with hot water. Discard this filtrate. Burn off the paper at under 500° and add the residue to the ignited cupferron precipitate at the next stage.

Cool the solution to under 20° and add a cold, freshly prepared, 6 per cent solution of cupferron with constant stirring until the precipitate is reddish brown. Addition of cupferron beyond that point will only contaminate the precipitate with more iron. Mix ashless paper pulp with the precipitate and filter through a rapid paper. Wash thoroughly with cold 1:9 hydrochloric acid, usually 12-15 times. Dry the paper and contents in platinum, usually a crucible, and ignite at under 500° until the carbon is burned off. Add the ash from copper separation at this point.

If the steel does not contain appreciable vanadium, omit the directions given in this paragraph. Add 5 ml. of 48 per cent hydrofluoric acid and 10 ml. of 70 per cent perchloric acid and evaporate to less than 5 ml. Cool, dilute to about 50 ml., neutralize with 10 per cent sodium hydroxide solution, and add 5 ml. in excess. Boil for a few minutes, let settle, and filter on a hard paper. Wash the precipitate well with hot water and discard the filtrate and washings. Dry the paper and contents in a platinum crucible and burn off the carbon at under 500°.

Whether or not vanadium was removed, an ash is now available in platinum. Fuse the contents of the crucible with one gram of potassium pyrosulfate and dissolve the cooled melt in 25 ml. of 1:9 sulfuric acid. Dilute to a suitable volume with 1:9 sulfuric acid and add 85 per cent phosphoric acid, drop by drop, until the iron color is removed. Develop with hydrogen peroxide.

Insoluble Titanium in Titanium Steel.[7] Weigh 5.0 grams of sample and add 100 ml. of 2:1 hydrochloric acid. Heat, but do not boil, on a hot plate until the reaction has ceased. Add a small wad of ashless filter pulp and filter. Wash well with hot water. Ash the residue in a platinum crucible in a muffle furnace. Cool and add 1 ml. of 1:1 sulfuric acid, 3 ml. of water, and 5 ml. of 48 per cent hydrofluoric acid. Evaporate to dryness on a sand bath. Add sufficient sodium bisulfate to take all the residue into fusion. Heat gently over a flame until the melt is clear and uniform. Cool and add to 20 ml. of 1:1 sulfuric acid. Warm to dissolve the melt, cool, and transfer to a 250-ml. volumetric flask. If colored by iron salts, add 5 ml. of 85 per cent orthophosphoric acid. Dilute to volume and use an aliquot for development with hydrogen peroxide.

[7] "Methods of Titanium Alloy Manufacturing Company," p. 5. (1943.)

Alternatively,[8] dissolve a 1-gram sample in 25 ml. of 1:1 hydrochloric acid by moderate heat for 1 hour. All action should have ceased by that time. Filter and wash with 1:20 hydrochloric acid. The filtrate contains the titanium originally present in metallic form.

Return the insoluble residue and paper to the original beaker and add 7 ml. of 72 per cent perchloric acid and 30 ml. of concentrated nitric acid. Digest at a moderate temperature until all organic matter has been destroyed, then heat to vigorous fumes for 3 minutes. Cool, add 25 ml. of water and 3 drops of saturated sulfurous acid, and filter. Use the filtrate directly as sample or aliquot. Develop the color with hydrogen peroxide.

Carbon Steel, Open-hearth Iron, and Wrought Iron Containing Less than 0.05 Per Cent of Titanium. Weigh out a 5-gram sample and heat gently with 150 ml. of 1:4 hydrochloric acid until reaction is complete. Continue as for titanium steel starting at "Cool the solution to under 20° and . . ."

Alloy Steels.[9] Prepare an acid mixture containing 40 ml. of concentrated orthophosphoric acid and 12 ml. of concentrated sulfuric acid per 100 ml. Dissolve a 0.08-0.7 gram sample in 30 ml. of this mixture, 10 ml. of concentrated hydrochloric acid, and 5 ml. of concentrated nitric acid. Evaporate to fumes, heat for five minutes, and let cool. Take up in water and dilute to about 80 ml. Add 15 ml. of 12.5 per cent stannous chloride in 1:9 hydrochloric acid and dilute to 100 ml. Use an aliquot for determination by hydroquinone.

Alternatively,[10] heat 1 gram of alloy gently with 80 ml. of 1:3 sulfuric acid. When reaction ceases, add 10 ml. of concentrated nitric acid. Boil to fumes of sulfur trioxide and let cool. Add 20 ml. of 1:3 sulfuric acid and after mixing well add 75 ml. of water. When warmed to full solution, filter through paper pulp and wash with 1:20 sulfuric acid. Cool and dilute to 200 ml. Develop an aliquot by hydrogen peroxide, using the undeveloped sample solution as blank.

Chromium-nickel and Chromium-molybdenum Steel.[11] Dissolve a 1-gram sample in 9 ml. of concentrated hydrochloric acid and 3 ml. of

[8] Alfred Weissler, *Ind. Eng. Chem., Anal. Ed.* **17,** 775-7 (1945).

[9] Charles M. Johnson, *Iron Age* **157,** No. 14, 66-9 (1946).

[10] F. W. Haywood and A. A. R. Wood, "Metallurgical Analysis by Means of the Spekker Absorptiometer," pp. 68-71. Adam Hilger's Sons, London, England (1943).

[11] Louis Silverman, *Ind. Eng. Chem., Anal. Ed.* **14,** 791-2 (1942).

concentrated nitric acid. Add 30 ml. of 72 per cent perchloric acid and heat until the chromium is oxidized to the red trioxide, then heat 5 minutes more. Cool and filter through a Gooch or fritted-glass crucible. Wash with five 5-ml. portions of 72 per cent perchloric acid which has been heated to fumes and cooled. Wash the contents of the crucible, consisting largely of chromic oxide, into the original reaction beaker with water. Add 15 ml. of 72 per cent perchloric acid and heat to fumes. Cool, filter into a 100-ml. volumetric flask, and wash 5 times as before with 5-ml. portions of 72 per cent perchloric acid which has been heated to fumes and cooled. Dilute the combined washings and filtrate to volume and use an aliquot as sample.

This method is particularly advantageous for the 5Cr-0.6Mo type of steels, giving a colorless solution. Some titanium may be retained by the substantial amount of chromium trioxide. With 18Cr-8Ni there will be some nickel in the filtrate, best compensated by addition to the standard or by reading of absorption in a narrow wave band.

Chromium-molybdenum-nickel Steel.[12] Heat a 1-gram sample with 12 ml. of 1:1 hydrochloric acid and 4 ml. of 1:1 nitric acid until solution is complete. Add 30 ml. of 72 per cent perchloric acid and heat to fumes of perchloric acid. Filter and wash as in the preceding method. Evaporate the filtered solution of sample to 10-15 ml. and add 25 ml. of water. Heat to boil off residual chlorine and let cool. Dilute to a known volume for use of aliquots or use the entire sample.

High-chromium, Corrosion-resistant Steel.[13] To a 5-gram sample, or less if the titanium content is over 0.05 per cent, add 24.8 ml. of concentrated hydrochloric acid and 47.6 ml. of water. Stir continuously while adding 7.6 ml. of concentrated sulfuric acid. Warm gently to dissolve, then digest on a hot plate to reduce the volume. Transfer to a sand bath and continue heating until the residue is almost dry, but do not bake. Cool and dissolve in 150 ml. of hot water. Cool in running water to below room temperature. Add a ball of filter pulp about 0.5 inch in diameter and stir well Add fresh 6 per cent cupferron solution dropwise until the precipitate begins to turn a deep reddish brown. This will usually require 3-5 ml. Stir for 2-3 minutes and allow to settle in a cold water bath for 5 minutes.

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[12] *Ibid.*

[13] Oscar Milner, Kenneth L. Proctor, and Sidney Weinberg, *Ind. Eng. Chem., Anal. Ed.* 17, 142-5 (1945).

Filter through a paper containing about half the previous amount of paper pulp, wash with water, then wash with ten 10-ml. portions of 1:20 sulfuric acid. Transfer the paper and precipitate to the original beaker and add 25 ml. of concentrated nitric acid, then 10 ml. of concentrated sulfuric acid and 10 ml. of 72 per cent perchloric acid. Evaporate to fumes of sulfur trioxide to destroy organic matter. Cool, dilute to 75 ml., and warm gently to dissolve soluble salts. Cool and, if molybdenum is less than 0.05 per cent and vanadium is absent, the ensuing peroxide separation procedure may be omitted.

If either molybdenum or vanadium is present, make the solution of soluble salts just alkaline with 25 per cent sodium hydroxide solution. Add, with continuous stirring, 0.5 gram of sodium peroxide in small portions. When the reaction has subsided, bring to the boiling point and boil for 10 minutes. Cool and filter through a paper containing some pulp. Wash with 1:99 ammonium hydroxide until the washings are colorless. Transfer the paper and precipitate to the original beaker, add 15 ml. of concentrated nitric acid, 15 ml. of concentrated sulfuric acid, and 5 ml. of 72 per cent perchloric acid. Heat until the organic matter is destroyed and evaporate to strong fumes of sulfur trioxide. Cool, dilute to 75 ml., and warm if necessary to dissolve soluble salts.

To this solution in the cold or to the molybdenum- and vanadium-free solution add 3 ml. of 6 per cent sulfurous acid and boil for 5 minutes to expel excess sulfur dioxide. Add 10 drops of 1:1 nitric acid to the boiling solution and boil for 1-2 minutes. Cool and filter into a 100-ml. volumetric flask. Dilute to volume and use 10-ml. portions as aliquots.

Highly Refractory Steel.[14] Dissolve a 1-gram sample in 50 ml. of 1:4 sulfuric acid, and during the solution add dropwise 2 ml. of concentrated nitric acid to oxidize the sample. Evaporate to sulfur trioxide fumes, let cool, and add 100 ml. of water. Filter and wash the filter well with 1:12 hydrochloric acid. Titanium may be in both the precipitate of silica and the filtrate. Ignite the precipitate and paper in platinum. Cool and add 1 ml. of 48 per cent hydrofluoric acid. Add a few drops of concentrated sulfuric acid and heat to volatilize the silica. Let cool and add 2 grams of potassium pyrosulfate. Fuse, let cool, dissolve in water, filter, and add the filtrate to that previously obtained. Discard the residue on the filter.

Add 1.5 ml. of fresh 3 per cent cupferron solution dropwise, with vigorous stirring, to precipitate iron and titanium. After sedimenting

[14] L. V. Gal'perin, *Zavodskaya Lab.* **11**, No. 1, 105-8 (1945).

for 10-15 minutes, filter and wash very thoroughly with 1:20 sulfuric acid, then with 1:20 ammonium hydroxide. This insures complete removal of tungsten and molybdenum, the two elements that offer the most serious interference. Dry the paper and contents and ignite. Let cool and fuse with 2 grams of mixed sodium and potassium carbonates. Take up the cooled melt in 20 ml. of hot water and add 1 ml. of 25 per cent sodium hydroxide. Bring to a boil and filter. Vanadium is now in solution, iron is on the filter as the oxide or hydroxide, titanium as sodium titanate. Wash the filter well with 1 per cent sodium carbonate solution, dry, and ash in platinum. Take up the ash by fusion with 2 grams of potassium pyrosulfate. Take up the melt in 65 ml. of 1:10 sulfuric acid, warming if necessary to get a clear solution. Use all or dilute to a known volume and use an aliquot as sample.

Ferrosilicon. Dissolve 5 grams of the alloy in a 1:1 mixture of concentrated nitric acid and 48 per cent hydrofluoric acid. Add 10 ml. of concentrated sulfuric acid and evaporate to sulfur trioxide fumes. Cool, moisten with water, and add more 48 per cent hydrofluoric acid to volatilize the silica. Ignite strongly. Dissolve the residue in 50 ml. of 1:1 hydrochloric acid and dilute to 300 ml. with hot water. Add a few drops of bromine water and a slight excess of 1:1 ammonium hydroxide. Boil for 1-2 minutes, filter, and wash the precipitate with hot water. Dissolve in hot 1:3 hydrochloric acid. Reprecipitate with 1:1 ammonium hydroxide, boil, and filter through the same filter paper. The precipitate contains aluminum, iron, titanium, and manganese.

Dissolve the precipitate in hot 1:3 hydrochloric acid and evaporate the solution to a sirup. Cool and extract the iron with ether (page 304). Aluminum, titanium, and chromium are in the acid solution. Evaporate ether from the acid solution and add 1:1 ammonium hydroxide until a faint permanent precipitate is formed. Redissolve this in 1:1 hydrochloric acid, using 2-3 drops of excess acid. Heat to boiling and add a few drops of 10 per cent ammonium bisulfite solution. Then add a few drops of 1 per cent phenylhydrazine solution. Filter and wash with cold water. Ignite the precipitate and fuse with 1 gram of potassium bisulfate. Cool and dissolve in 50 ml. of 1:20 sulfuric acid. Transfer to a 100-ml. volumetric flask and dilute to volume with 1:20 sulfuric acid.

Aluminum-copper-nickel-manganese-iron Alloys. The sample solution was prepared for copper determination (page 80). Use the peroxide method.

Tantalum Metal.[15] Decompose 50 mg. of powdered metal in a platinum crucible with 10 ml. of 1:4 nitric acid and a few drops of 48 per cent hydrofluoric acid. Add a few drops of sulfuric acid and evaporate the contents of the crucible to dryness and the complete removal of sulfur trioxide. Fuse the residue to a clear melt with 2 grams of potassium pyrosulfate and cool. Take up with 1 gram of oxalic acid and some warm water and warm until a clear solution results. Transfer the hot contents of the crucible to a 50-ml. volumetric flask, cool, and dilute to volume. This method of preparation lends itself to subsequent determination of titanium by means of the chromotropic acid reagent.

Alternatively, fuse 0.5-1.0 gram of powdered sample with 2 grams of potassium pyrosulfate. Dissolve the cooled melt in 30 ml. of concentrated sulfuric acid, add 8 grams of succinic acid, and dilute the solution with water. Treat with 30 ml. of 85 per cent phosphoric acid, dilute to a known volume, and use an aliquot as sample.

Unsintered Metal Carbides. The preparation of a solution was described for determination of iron (page 287). Use an aliquot of the same solution for determination of titanium.

Tin Concentrates.[16] Mix 1 gram of the sample with 4 grams of sodium carbonate and 8 grams of sodium peroxide in a nickel crucible. Heat the crucible gently at first, then fuse the charge, and continue for 2 minutes after complete fusion has occurred. Cool and leach the fusion with 100 ml. of water. Add 65 ml. of concentrated hydrochloric acid and agitate the solution until the melt has completely dissolved. Remove the crucible and rinse with 1:3 hydrochloric acid. Add the washings to the solution and heat to expel chlorine. Adjust the acidity to that of 1:20 hydrochloric acid. Pass in hydrogen sulfide for 30 minutes and allow the precipitate to settle. Filter and wash the precipitate with warm 1:33 sulfuric acid containing hydrogen sulfide. Expel the hydrogen sulfide from the filtrate by boiling, then oxidize the solution with 3 per cent hydrogen peroxide. Render the solution alkaline with 1:1 ammonium hydroxide, heat to boiling, and filter. Wash the precipitate with 1:20 ammonium hydroxide containing about 0.1 per cent of ammonium chloride Discard this filtrate and washings.

Pour 150 ml. of 1:30 hydrochloric acid through the filter to dissolve the iron, aluminum, and titanium precipitates. Again precipitate by

[15] P. Klinger, E. Stengel and H. Wirtz, *Metall u. Erz* 38, 124-7 (1941).

[16] Silve Kallmann, *Ind. Eng. Chem., Anal. Ed.* 15, 166-74 (1943).

addition of an excess of 1:1 ammonium hydroxide, *heat to boiling*, and filter. Wash as before with ammonium hydroxide containing ammonium chloride. Again dissolve the washed precipitate in 150 ml. of 1:30 hydrochloric acid. Add two grams of tartaric acid and pass in hydrogen sulfide for 20 minutes. Filter any precipitate and wash with 1:100 sulfuric acid containing hydrogen sulfide and about 0.1 per cent of tartaric acid. Discard the precipitate and make the solution alkaline with 1:1 ammonium hydroxide, adding 10 ml. in excess. Pass in hydrogen sulfide for 5 minutes and filter immediately. Wash the precipitate with an ammonium sulfide solution containing 0.1 per cent of tartaric acid. Discard this precipitate of iron.

Boil the filtrate containing the titanium and aluminum to expel ammonium sulfide, add 40 ml. of 1:1 hydrochloric acid, and boil again to remove hydrogen sulfide. Add 30 ml. of 3 per cent hydrogen peroxide and boil to decompose excess peroxide. Dilute to 300 ml., cool, and add 6 per cent cupferron solution until no further precipitate forms. This precipitate contains titanium, zirconium, some columbium, and tantalum. Pour through a filter containing paper pulp and wash with 1:9 hydrochloric acid. Ignite the precipitate in a platinum crucible, fuse with sufficient sodium bisulfate to dissolve the residue completely, and cool. Add 1:10 sulfuric acid to dissolve, transfer to a 100-ml. volumetric flask, and make up to volume with 1:10 sulfuric acid. Develop the color due to titanium in an aliquot of the solution by hydrogen peroxide.

Glass and Silicate Minerals. Fuse 1 gram of pulverized sample with 5 grams of sodium carbonate. Cool, extract the fused mass with hot water, then add 1:1 hydrochloric acid gradually until in excess. Evaporate to dryness and moisten with concentrated hydrochloric acid. Add a few drops of water and heat on the water bath for 10-30 minutes. Add water and filter. The precipitate is mostly silica but retains some iron and titanium. Volatilize the silica by treating the precipitate in a platinum crucible with a few drops of 1:1 sulfuric acid, then adding a few ml. of 48 per cent hydrofluoric acid. Evaporate and ignite strongly. Repeat the evaporations with sulfuric acid several times.

To the hot filtrate from the precipitate of silica add 1:3 ammonium hydroxide to faint alkalinity. Filter and wash the precipitate with a 2 per cent ammonium chloride solution. Transfer to the crucible containing the titanium and iron left after the volatilization of silica. Fuse the combined precipitates with about 5 grams of sodium carbonate for 2-3 hours, let cool, and transfer to a beaker with 200-250 ml. of boiling water. Heat on the water bath until disintegration is complete. Filter

off the iron and titanium oxides, wash, dry, and fuse again with sodium carbonate, finally collecting the iron and titanium precipitate free of other metals.

Fuse the mixed oxides with 15-20 parts of potassium acid sulfate until completely dissolved. Let cool with the crucible covered. Transfer to a beaker with about 75 ml. of boiling 1:20 sulfuric acid. Heat on the water bath until solution is complete. Transfer to a 100-ml. volumetric flask, let cool, and add 5 ml. of a concentrated solution of potassium persulfate. Add 1:20 sulfuric acid until a total volume of 95 ml. is obtained. This oxidizes ferrous to ferric ion but does not oxidize titanium. Dilute to volume and use an aliquot for development with hydrogen peroxide.

There are various alternative methods of preparation applicable under varying conditions which depend on the nature of the sample. As one method, treat 0.5 gram of finely ground sample with 1 ml. of 1:1 sulfuric acid and 10 ml. of 48 per cent hydrofluoric acid. Evaporate to dryness and ignite. Cool and pulverize. Fuse with 4 grams of potassium pyrosulfate for 10 minutes at a temperature at which sulfur trioxide fumes are not given off. Heat further to an orange color, avoiding loss of sulfur trioxide as far as possible. Powder the residue and disperse in 50 ml. of water. Filter into a 100-ml. volumetric flask and wash with water until a volume of 90 ml. is obtained. Dilute to volume and use an aliquot.

Another method [17] of working up the sample is to fuse a 0.1-gram sample of silicate with 4 grams of mixed sodium carbonate and potassium carbonate. Dissolve the melt in 10 ml. of 1:2 hydrochloric acid. Add 1:1 ammonium hydroxide to faint alkalinity to precipitate aluminum, iron, and titanium as hydroxides. Filter and wash well. Then take up in excess of 1 per cent oxalic acid. When solution is complete, titrate back to neutrality to methyl red with 0.4 per cent sodium hydroxide solution. Transfer to a 100-ml. volumetric flask, add 20 ml. of 1:1 sulfuric acid, and dilute to volume.

Another technic is the following. Fuse a 1-gram sample with 5 grams of anhydrous sodium carbonate. Extract the melt with 50 ml. of boiling water containing a few drops of alcohol to reduce manganates, and filter. The titanium is in the residue as hydrolysed sodium titanate. Wash the residue with 2 per cent sodium carbonate solution and discard the filtrate. Dry the residue, burn off the paper, and fuse with an excess

[17] A. K. Babko, *Zavodskaya Lab.* 4, 891-3 (1935); M. F. Chigrin, *ibid.* 6, 758 (1937).

of potassium bisulfate. Dissolve the melt in 1:9 sulfuric acid and dilute to a known volume. To an aliquot add 5 ml. of 85 per cent orthophosphoric acid and dilute to a suitable volume for the hydrogen peroxide method. Use the same amount of phosphoric acid in the same volume of standard.

For microdetermination [18] take 0.05 gram or less. Moisten in a small platinum crucible with 3 drops of water, 6 drops of 48 per cent hydrofluoric acid, and 6 drops of 1:1 sulfuric acid. Vaporize the silica and hydrofluoric acid and heat to sulfur trioxide fumes. Add 4 drops of water and evaporate again. Take up with water and use as a sample by the hydrogen peroxide method.

Quartzite.[19] Fuse 0.5 gram of sample with 2 grams of potassium pyrosulfate. Cool the melt and extract with boiling water. Filter and discard the filtrate. Dissolve the residue by heating with 20 ml. of 1:3 sulfuric acid. Filter, catch the filtrate in a 100-ml. volumetric flask, and dilute to volume.

Rutile and Iron Ores. Moisten a 1-gram sample in a platinum crucible with water. Add a few drops of concentrated sulfuric acid and 1 ml. of 48 per cent hydrofluoric acid. Heat until sulfur trioxide fumes are no longer given off. Repeat the evaporation with sulfuric acid several times. Add 10 grams of sodium carbonate and 0.2 gram of sodium nitrate or sodium peroxide. Fuse for at least 30 minutes. Cool and heat the crucible and cover with hot water until the melt is disintegrated. Iron oxide and sodium titanate remain as insoluble material. Remove the crucible and dissolve any residue with hot 1:1 hydrochloric acid. Filter the contents of the beaker and wash with hot water. Dissolve the precipitate in 1:1 hydrochloric acid and add the acid washings from the crucible. Heat until solution is complete, and a volume of 20 ml. remains. Cool, add 2 ml. of concentrated hydrochloric acid, and transfer to a separatory funnel. Rinse the beaker with 10 ml. of 1:1 hydrochloric acid and add to the solution. Add an equal volume of peroxide- and alcohol-free ether saturated with concentrated hydrochloric acid. Shake and let stand for 10 minutes. Draw off the aqueous layer and repeat the ether extraction until the ether layer is no longer green. Rinse the ether layers with 10 ml. of 1:1 hydrochloric acid and add this to the aqueous portion. Heat the acid solution over a steam bath

[18] I. P. Alimarin and A. Ya. Sheskol'skaya, *ibid.* 11, 141-5 (1945).
[19] N. O Zeldin and Z. V. Ogur, *Ogneupory* 6, 1702-3 (1938).

to expel the ether and cool. Use as sample or dilute to a known volume and use an aliquot.

Corundum.[20] Fuse a 4-gram sample with 8 grams of anhydrous sodium carbonate and 4 grams of borax. Cool and add 15-20 ml. of 1:6 hydrochloric acid to dissolve. Transfer to a 100-ml. volumetric flask and dilute to volume. An alternative is fusion with potassium bisulfate.[21]

Pigments.[22] To 1 gram of sample add 5 grams of potassium bisulfate and fuse to a clear melt in a muffle. Cool and dissolve the residue in 1:3 sulfuric acid. Transfer to a 100 ml. volumetric flask and dilute to volume.

Bauxite.[23] Fuse a 0.1-gram sample with excess potassium persulfate. Take up the melt in 1:9 sulfuric acid and determine titanium by the peroxide method.

Portland Cement.[24] Separate the iron and aluminum as usual as hydroxides. Fuse this precipitate with potassium persulfate and take up in 1:9 sulfuric acid. Use the peroxide method on this solution.

Soap.[25] Weigh a sample to contain 2-4 mg. of titanium dioxide and add 120 ml. of 95 per cent ethanol. Boil for 3 minutes, allow to settle, and filter by decantation. Digest the residue again with alcohol and refilter. Wash the filter with alcohol and ash. Add 20 grams of ammonium sulfate and 20 ml. of concentrated sulfuric acid, stir, and heat over a burner for 5-10 minutes to dissolve the titanium. Cool, dilute, and filter into a 250-ml. volumetric flask. Dilute to volume and aliquot.

Plant and Animal Tissue. While the amounts of titanium present in such tissue are minor, of the order of 0.003-0.008 mg. per 100 grams of sample,[26] occasion for its determination does arise. Ash[27]

[20] A. N. Miklashevskii, *Zavodskaya Lab.* 6, 1209-13 (1937).
[21] J. Raffin, *Ann. chim. anal.* 25, 56-7 (1943).
[22] H. Ginsberg, *Metallwirtschaft* 16, 1107-12 (1937).
[23] J. Jaime Puértolas, *Ion* 5, 276-8 (1945).
[24] J. J. Diamond, *Rock Products* 49, No. 4, 103-4, 124 (1946).
[25] L. B. Parsons and F. A. Vaughan, *Oil and Soap* 18, 64-5 (1941).
[26] Sh. E. Kaminskaya, *Trav. lab. biogéochim. acad. sci.* U.R.S.S. 4, 227-44 (1937)
[27] Louis C. Maillard and Jean Ettori, *Compt. rend.* 202, 594-6 (1936).

a 50-gram sample below 500°. Take up the ash with 10 ml. of 1:1 sulfuric acid and heat to fumes of sulfur trioxide. Let this cool and add about 60 ml. of water. Add 1 gram of tartaric acid and a drop of 10 per cent ferric chloride solution. Add 5 per cent cupferron solution until precipitation is complete and filter. Wash the residue on the paper with 1:18 sulfuric acid containing 0.2 per cent of cupferron. Discard the filtrate and washings.

Ignite the cupferron precipitate and cool. Fuse the ash with 2 grams of potassium bisulfate. Let cool and take up the melt in 10 ml. of 1:18 sulfuric acid. Add 0.1 gram of tartaric acid, then 1:1 ammonium hydroxide until nearly neutralized. Saturate the solution with hydrogen sulfide and gradually add 1:1 ammonium hydroxide until alkaline. Filter the precipitated ferrous sulfide and wash with 1:100 ammonium hydroxide containing hydrogen sulfide. Discard the paper and residue. Dilute the filtrate to about 50 ml. and add 10 ml. of 1:1 sulfuric acid. Boil until hydrogen sulfide is removed.

Add a drop of solution of a zirconium salt to the solution and then 5 per cent solution of cupferron until precipitation is complete. Filter and wash the precipitate with 1:18 sulfuric acid containing 0.2 per cent of cupferron. Discard the filtrate and washings. Ash the paper and fuse the residue with 0.2 gram of potassium bisulfate. Take up the melt in 10 ml. of 1:9 sulfuric acid and use as sample.

Removal of Iron as Fluoride. This has been described for separation from copper (page 107). For separation from titanium adjust the acidity to contain 3 ml. of concentrated sulfuric acid per 100 ml. When the separated precipitate was redissolved and reprecipitated there was no loss of titanium.

STANDARDS

From Potassium Titanium Oxalate. Transfer 2.2124 grams of recrystallized potassium titanium oxalate, $K_2TiO(C_2O_4)_2 \cdot 2H_2O$, to a flask. Add 8 grams of ammonium sulfate and treat with 100 ml. of concentrated sulfuric acid. Gradually heat to boiling and boil for 5-10 minutes. Cool, dilute to 300 ml., and transfer to a liter volumetric flask. Dilute to volume. Each ml. contains approximately 0.3 mg. of titanium, or 0.5 mg. of titanium dioxide.

From Potassium Titanium Fluoride. Weigh 2.70 grams of recrystallized potassium titanium fluoride, $K_2TiF_6 \cdot H_2O$, into a platinum dish. Add 100 ml. of concentrated sulfuric acid and heat to strong sulfur

trioxide fumes. Let cool and wash down the sides of the dish with water. Heat to strong sulfur trioxide fumes again. Repeat the cooling, washing down, and heating. Finally pour the solution rapidly with stirring into 900 ml. of water. If made properly, it will be perfectly clear. Cool, dilute to 1 liter, and preserve in a glass-stoppered bottle. Each ml. of the solution should contain 0.5 mg. of titanium or 0.833 mg. of titanium dioxide. If in doubt, standardize by precipitation of 50-ml. portions diluted to 250 ml. by addition of 1:1 ammonium hydroxide to the boiling solution. Filter, ignite at about 1200°, and weigh as titanium oxide.

From Titanic Acid. Fuse 1.05 grams of ignited pure titanic acid with 10 grams of sodium carbonate. Digest with 100 ml. of hot water until the soluble alkali is completely dissolved. Filter and wash the insoluble sodium titanate with hot 0.01 per cent sodium carbonate solution. Dissolve the titanate from the filter with 100 ml. of 1:1 sulfuric acid and dilute to 1 liter with water. One ml. of this solution contains about 0.6 gram of titanium. Remove an aliquot and determine the titanium gravimetrically as follows.

Add concentrated ammonium hydroxide until a precipitate appears which redissolves on stirring. Add 1:50 ammonium hydroxide until a faint permanent precipitate is obtained. Dissolve this by adding 15 ml. of 1:3 hydrochloric acid. Add 100 ml. or more of 20 per cent sodium thiosulfate solution and stir until free sulfur begins to separate. Boil for 10 minutes and filter. Wash with 1:50 acetic acid, ignite and weigh the titanium dioxide. From this weight calculate the amount of titanium sulfate in the standard solution.

From Titanium Dioxide. Prepare ammonium bisulfate by mixing chemically equivalent quantities of ammonium sulfate and sulfuric acid. Fuse 1 gram of pure titanic oxide with about 10 grams of the above. Take up with 200 ml. of 1:4 sulfuric acid and dilute to 1 liter with water. Standardize by evaporation of a measured portion to dryness in platinum and ignite to constant weight. If necessary add ammonium carbonate to assist in volatilizing sulfuric acid.

Titanium by Hydrogen Peroxide

When a titanium sulfate solution is treated with hydrogen peroxide a yellow color is formed.[28] The presence of 2.5 ppm. of titanium is

[28] A. Weller, *Ber.* 15, 2592-9 (1882).

sufficient to produce the coloration,[29] and the color is stable for over two years. The reaction is often assumed to be due to the formation of pertitanic acid, H_4TiO_5, but other data indicate the formation of the $[TiO_2(SO_4)_2]^=$ ion. In the presence of excess hydrogen peroxide, Beer's law holds up to[30] a titanium concentration of 50 ppm.

A desirable standardization is to develop in 1:9 sulfuric acid with sufficient hydrogen peroxide to give a 1 per cent concentration. After development of color it may, if necessary, be diluted with 1:9 sulfuric acid. The acidity is necessary to avoid hydrolysis. The peak of absorption is then at 410 mμ. If orthophosphoric acid is also present, the peak is narrower and at about 400 mμ.[31] If a mercury arc is used, the maximum absorption occurs at 436 mμ and 546 mμ.[32] The spectrophotometric method improves both sensitivity[33] and selectivity.

A preliminary treatment with cupferron, the ammonium salt of nitrosophenylhydroxylamine, permits the separation of titanium from nickel, chromium, and most of the interfering iron. Zirconium, copper, and vanadium are coprecipitated in the cupferron reaction, but copper dissolves when the precipitate is washed with ammonium hydroxide, and the zirconium does not interfere with the subsequent peroxide color reaction. Vanadium is only partially removed by washing with ammonium hydroxide, so that fusion of the ignited cupferron residue with sodium carbonate to yield the soluble sodium vanadate may be necessary. A single precipitation may not effect complete separation from large quantities of these metals. In the presence of iron only, the cupferron precipitation may be avoided by decolorizing the iron with orthophosphoric acid.[34] Chromium in amounts up to 1 per cent does not interfere in the oxidation of an orthophosphoric acid solution of the sample.[35] Color due to iron may be filtered out by reading in the green band, but sensitivity is greatly reduced.

Various methods are used for separation of large amounts of chromium. One is by the lead perchlorate method.[36] Another makes use of the fact that chromium trioxide is quantitatively insoluble in cold 69-72

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[29] B. Bagshawe, *J. Soc. Chem. Ind.* 57, 260-5 (1938).

[30] Maximiliane Bendig and H. Hirschmüller, *Z. anal. Chem.* 120, 385-93 (1941).

[31] Alfred Weissler, *Ind. Eng. Chem., Anal. Ed.* 17, 775-7 (1945).

[32] H. Pinsl, *Angew. Chem.* 50, 115-20 (1937).

[33] I. Sokolova, *Aviapromyshlennost* 1939, No. 3, 52-5.

[34] M. D. Kenigstuhl, *Zavodskaya Lab.* 9, 1203 (1940).

[35] S. M. Gutman, E. N. Zarogatskaya and Z. A. Vyrapaeva, *ibid.* 9, No. 1, 101-2 (1940).

[36] U. S. Steel Chemists Committee, "Sampling and Analysis of Carbon and Alloy Steels," p. 62, Reinhold Publishing Corp., New York (1938).

per-cent perchloric acid,[37] wherein small amounts of titanium are soluble. Another is electrolysis using a mercury cathode.[38] Yet another[39] is volatilization of chromic chloride from boiling perchloric acid to which sodium chloride has been added.

The other interfering metals must be present in relatively large concentrations to produce an appreciable effect. Nickel in amounts up to 2 per cent and molybdenum up to 1 per cent may be present without introducing any significant error. At 1.5 per cent of molybdenum the positive error is equivalent to less than 0.02 per cent. The presence of halogen ions also introduces an interference,[40] removable by silver nitrate precipitation.[41] Color in the solution before addition of hydrogen peroxide may be allowed for in the blank setting of the photometer, provided it will not be altered by hydrogen peroxide.

Aside from the colors which are inherently present from some ions there are others which give color reactions with hydrogen peroxide. The principal ones are vanadium, uranium, molybdenum, and columbium. Of these only vanadium has an appreciable effect on results obtained by the spectrophotometer. By use of a narrow wave band results for titanium and vanadium are obtained on the same solution at 400 or 410 and 460 mμ and solved algebraically. By introduction of a value at 330 mμ molybdenum can also be determined at the same time. Readings at 436 and 546 mμ are also used for titanium and vanadium.[42]

It is often desirable to work in solutions containing perchloric acid. The relatively minor effect on the color developed is shown in Table 5.[43] The presence of large amounts of alkali sulfates reduces the color intensity. A trace of fluoride bleaches the color. Citric acid bleaches the color but tartaric acid does not. In 6 N acid the color is slightly less intense than in 1.5 N. The intensity increases with temperature. As a variant, if a sample is fused with sodium peroxide in the melt, and the melt taken up with water and acidified, the hydrogen peroxide produced in the melt develops the desired peroxide reaction.

Procedure. The usual reagent is 3 per cent hydrogen peroxide. This can also be prepared in the laboratory from sodium peroxide. Add 7

[37] Louis Silverman, *Ind. Eng. Chem., Anal. Ed.* 14, 791-2 (1942).
[38] A. V. Endredy and Fr. Brugger, *Z. anorg. allgem. Chem.* 249, 263 (1942).
[39] Z. S. Mukhina, *Zavodskaya Lab.* 8, 162 (1939).
[40] H. Ginsberg, *Metallwirtschaft* 16, 1107-12 (1937); *Angew. Chem.* 51, 663-7 (1938).
[41] H. A. Liebhafsky, *Z. anal. Chem.* 105, 113-14 (1936).
[42] H. Pinsl, *Angew. Chem.* 50, 115-20 (1937).
[43] Alfred Weissler, *Ind. Eng. Chem., Anal. Ed.* 17, 695-8 (1945).

grams of sodium peroxide in small portions to 25 ml. of 1:1 sulfuric acid. After each addition let the solution cool to room temperature. Dilute with water to 100 ml.

TABLE 5. INFLUENCE OF PERCHLORIC ACID CONCENTRATION ON TITANIUM-PEROXIDE COLOR. BECKMAN SPECTROPHOTOMETER, 1-CM. CELL, 5 mμ BAND

Ml. of 70% Perchloric Acid per 50 Ml.	*Optical Density of 1 Mg. of Titanium per 50 Ml. at 410 mμ.*	*Position of Absorption Peak, mμ.*
0	0.305	410
1	0.309	410
3	0.301	410
5	0.305	410
10	0.304	410
15	0.305	415
25	0.297	420
40	0.285	425
49	0.272	430

Duplication. Select an aliquot of sample containing about 10-50 mg. of titanium. To this add sufficient 1:1 sulfuric acid to bring the total of sulfuric and perchloric acids to 10 ml. Prepare a blank of slightly smaller volume containing the same amounts of salts, acid, etc., but no titanium. Add 3 ml. of 3 per cent hydrogen peroxide to each. Dilute the sample to 100 ml. Add a standard solution of titanium sulfate to the blank and finally adjust the volume with 1:9 sulfuric acid to match that of the sample. The standard may also be prepared in parallel with the sample, and comparison made by balancing.

Transmittance. Prepare the sample as for duplication, compare with the sample without addition of hydrogen peroxide as a blank, and read against a curve prepared from a similar composition.

Titanium and Vanadium Simultaneously.[44] Adjust the sample so that 10 ml. of 70 per cent perchloric acid is present in 50 ml. of sample. To read in the cell as a blank, use this solution with 1 drop of water added to the aliquot. For the color development add 1 ml. of

[44] Alfred Weissler, *Ind. Eng. Chem., Anal. Ed.* 17, 775-7 (1945).

30 per cent hydrogen peroxide to the same aliquot and read at 400 mμ and 460 mμ. Tbeu solve by the following equations.

$$x = \text{mg. of titanium}$$
$$y = \text{mg. of vanadinm}$$
$$x = 5.40\,D_{400} - 3.38\,D_{460}$$
$$y = 15.9\,D_{460} - 7.54\,D_{400}$$

Titanium, Vanadium and Molybdenum Present.[45] Measure an aliquot not exceeding 35 ml. into a 50-ml. flask. Add 10 ml. of 70 per cent perchloric acid and mix. Add 0.5 ml. of 3 per cent hydrogen peroxide, dilute to volume, and mix. Read the transmittance at 460 mμ for vanadium, that at 410 mμ for titanium, that at 330 mμ for molybdenum. Then apply the following derived equations:

$$x = \text{mg. of titanium}$$
$$y = \text{mg. of vanadium}$$
$$z = \text{mg. of molybdenum}$$
$$D_{330} = 0.065x - 0.020y + 0.208z$$
$$D_{410} = 0.304x + 0.074y + 0.024z$$
$$D_{460} = 0.205x + 0.100y + 0.001z$$
$$x = 7.01\,D_{410} - 0.57\,D_{330} - 5.46\,D_{460}$$
$$y = 20.7\,D_{460} - 14.3\,D_{410} + 1.55\,D_{330}$$
$$z = 5.12\,D_{330} - 3.57\,D_{410} + 3.67\,D_{460}$$

For maximum accuracy the wave bands must be not over 5 mμ and a spectrophotometer must be used. Wider wave bands may be necessary in some cases to permit sufficient light transmission, with necessarily lower accuracy. The comparative spectra are shown in Figure 20 to illustrate tbe significance of a narrow wave band.

Miscellaneous

The development of color by bydroquinone in strongly acid solution is a method of determination of titanium.[46] To a 4-ml. aliquot of the sample in strongly acid solution as reduced by stannous chloride add 40 ml. of a 5 per cent solution of hydroquinone in concentrated sulfuric acid. Hold at 70° for 3 minutes to develop the color. If tungsten or columbium is present, add 2 ml. of water and the color derived from them will fade. When 8 ml. of water are added, the color due to titanium will also fade.

[45] Alfred Weissler, *ibid.* 17, 695-8 (1945).

[46] Charles M. Johnson, *Iron Age* 157, No. 14, 66-9 (1946).

An intense yellow color is given by titanium and disodium-1,2-dihydroxybenzene-3,5-disulfonate.[47] The color intensity is independent of pH over the range 4.3-10. The system obeys Beer's law. Aluminum, calcium, and tungsten reduce the color intensity, but excess reagent overcomes this. Ferric iron, vanadium, and uranium develop colors with the reagent. That due to ferric ion disappears by using a 1:1 sodium acetate-acetic acid buffer whose pH is 4.7, and adding 0.05 gram of sodium hyposulfite, $Na_2S_2O_4$, per 100 ml. of final solution. This reduces the iron to the ferrous state, which does not affect the color. After 20 minutes turbidity may appear.

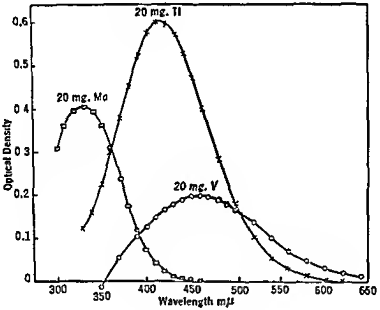

Fig. 20

Spectra of Peroxide Complexes of Molybdenum, Titanium and Vanadium

Iron and titanium may be determined simultaneously. At pH 4.7 determine the transmittance without hydrosulfite at 560 mμ which is the maximum for the iron. Then add hydrosulfite and measure the transmittance at 410 mμ.

The addition of thymol to a sulfuric acid solution of the titanium sample produces a red or reddish yellow color which is about 25 times as intense as that produced by the hydrogen peroxide method.[48] As usually applied, the method permits the determination of 0.2-2.5 mg. of titanium dioxide. The only substances likely to be present which

[47] John H. Yoe and A. Letcher Jones, *Ind. Eng. Chem., Anal. Ed.* 16, 111-15 (1944); John H. Yoe and Alfred R. Armstrong, *Science* 102, 207 (1945).

[48] Victor Lenher and W. G. Crawford, *J. Am. Chem. Soc.* 35, 138-45 (1913).

would alter the color are tungsten, molybdenum and chromium. The method is accurate to 5 per cent.

As reagent dissolve 5 grams of thymol in 5 ml. of glacial acetic acid and dilute with concentrated sulfuric acid to 100 ml. Protect this solution from sunlight. The proportion of thymol used to titanium dioxide should be at least 60:1 by weight. If the thymol is dissolved directly in the sulfuric acid, an interfering yellow color is produced. For the determination take an aliquot of sample in 1:10 sulfuric acid equivalent to about 1-10 mg. of titanium dioxide. Add 20 ml. of reagent and dilute to 100 ml. with concentrated sulfuric acid. If necessary take a 10-ml. aliquot, add 20 ml. of reagent, and dilute to 100 ml. with concentrated sulfuric acid. Compare with a standard titanium solution similarly treated at room temperature. Sample and standard must be at the same temperature, as increase of temperature decreases the color. At 100° it is destroyed. The dilution method is suitable for comparison, using concentrated sulfuric acid. When the acid content becomes lower than 79.4 per cent, the color becomes lighter than that proportional to the titanium content.

Alkaline titanates react with salicylic acid to form an intensely yellow salicylate.[49] Interference with the color developed occurs in the presence of very small amounts of fluorides, iron, molybdenum, zirconium, columbium and thorium. Tantalum pentoxide does not affect the color. Because of the interference of the common contaminants of titanic oxide, this method is of little value for commercial samples but may be used for an accurate determination of nearly pure titanic oxide. To an aliquot representing 5 grams of original sample add 5 grams of salicylic acid. Dilute to 500 ml. If the color is orange rather than yellow, remove 100 ml. and dilute to 1 liter. Compare with a series of standards. As little as 0.05 mg. of titanium per 100 ml. gives a faint yellow color distinguishable from a blank.

Quadrivalent titanium in 80-100 per cent sulfuric acid solutions reacts with aromatic phenols to give a blood-red color, not unlike that of ferric thiocyanate. As little as 0.2 ppm. can be determined.[50] The reaction with salicylic acid is also applied in such a sulfuric acid solution and is then about 5 times as sensitive as the peroxide method.

Many compounds containing the C(OH):C(OH) group give very sensitive color reactions with titanium, such as gallic acid,[51] ascorbic

[49] John Hughes Müller, *J. Am. Chem. Soc.* **33**, 1506-10 (1911).

[50] Max Schenk, *Helv. Chim. Acta* **19**, 625-39, 1127-35 (1936).

[51] Pabrita Nath Das Gupta, *J. Indian Chem. Soc.* 6, 855-63 (1929); F. M. Shemyakin and A. Neumolotova, *J. Gen. Chem.* (U.S.S.R.) 491-7 (1935).

acid,[52] and dihydroxymaleic acid.[53] For example, ascorbic acid does not lend any coloration to a dilute solution of titanium sulfate whose pH is less than 3.0. At a pH of 3.0 a yellow tinge appears which becomes an intense reddish brown as the pH increases to 4.6 and disappears completely at 5.2. In alkaline solution, a pale rose color becomes evident. The addition of sodium acetate to a solution of titanium which has been treated with gallic acid serves to intensify the color formed. Similar color changes are also produced by adrenaline, tyramine, tyrosine, hydroquinone, phenol, resorcinol, pyrogallol, phlorizin, or phloroglucinol.[54]. This clearly marks the development of the yellow color as a reducing reaction. Many metals interfere.

Sodium titanate gives a pale greenish yellow color with ammonium molybdate and nitric acid in the absence of phosphorus and silica.[55] Vanadium does not interfere. The color takes time to develop and does not fade in an hour. As a typical procedure, add 4 ml. of 5 per cent neutral ammonium molybdate solution and 2 ml. of 1:5 nitric acid to 50 ml. of neutral titanium solution. Dilute to 100 ml. and, after the color is fully developed, compare with a series of standards similarly prepared.

The addition of a 6 per cent chromotropic acid solution, 1,8-naphthol-3,6-disulfonic acid, to a solution of titanium results in the formation of a reddish brown color that can be measured photometrically by means of a blue light and a 470 mμ filter.[56] Iron does not interfere, and the reagent may be used for 0.3-3.5 per cent of titanium.[57] The method will determine 5-20 mg. of titanium per 100 ml. with a high degree of accuracy.

A reagent for iron, disodium-1,2-dihydroxybenzene-3,5-disulfonate has been applied[58] to detection of 0.01 ppm. of titanium. The tint and intensity are nearly constant for pH 4.3-9.6 and the yellow color conforms to Beer's law over a reasonable range. Reduction of iron to the ferrous state eliminates it as an interfering element.

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[52] Jean Ettori, *Compt. rend.* 202, 852-4 (1936).

[53] J. W. Mellor, *Trans. Eng. Ceram. Soc.* 12, Pt. I, 33-5 (1913).

[54] R. Roseman and G. Barac, *Compt. rend. soc. biol.* 140, Nos 17-18, 657 (1946).

[55] A. G. Woodman and L. L. Cayvan, *J. Am. Chem. Soc.* 23, 105 (1901).

[56] P. Klinger, E. Stengel and H. Wirtz, *Metall u. Erz* 38, 124-7 (1941); Cf. A. V. Endredy and Fr. Brugger, *Z. anorg. allgem. Chem.* 249, 263 (1942).

[57] P. Klinger and W. Koch, *Techn. Mitt. Krupp, Forschungsber.* 2, No. 14, 179-85 (1939); *Arch. Eisenhuttenwes.* 13, 127-32 (1940).

[58] John H. Yoe and Alfred R. Armstrong, *Anal. Chem.* 19, 100-2 (1947).

CHAPTER 23

ZIRCONIUM

Zirconium finds use mainly as an alloying element. The methods of colorimetric determination have a great deal in common with those for aluminum.

Sample

Steel.[1] Dissolve a 1-gram sample in 50 ml. of 1:1 hydrochloric acid. Dilute with water to 75 ml. and filter. Wash the residue with 3 portions of hot water, 3 portions of hot 1:1 hydrochloric acid, and 3 portions of water. Set the filtrate aside and ignite the paper and residue in platinum. Fuse the ash with a minimal amount of potassium hydrogen sulfate and cool. Dissolve the melt in 100 ml. of water and a few drops of concentrated sulfuric acid. Rinse the crucible and remove. Neutralize the solution to methyl orange by addition of concentrated ammonium hydroxide. Add a slight excess, boil, and filter. Wash the precipitate with water and discard the solution and washings. Pulp the paper and precipitate with 50 ml. of 1:1 hydrochloric acid in the original beaker. Heat to boiling for a minute and filter. Wash well with hot water. Combine this filtrate in a 500-ml. volumetric flask with that saved from the original solution of sample and discard the precipitate. Dilute to volume for the use of aliquots by the *p*-dimethylaminoazophenylarsonic acid method.

Standard

Dissolve 0.3533 gram of zirconium oxychloride, $ZrOCl_2 \cdot 8H_2O$, in 1:5 hydrochloric acid and dilute to 1 liter with the same acid. Each ml. contains 0.1 mg. of zirconium. If a high degree of accuracy is required, standardize gravimetrically by the conventional precipitation as the hydroxide with ammonium hydroxide and ignite to the dioxide.

Zirconium by Hydroxyanthraquinones

In the presence of an excess of strong acid, zirconium is one of the

[1] Walter G. Hayes and Edward W. Jones, *Ind. Eng. Chem., Anal. Ed.* 13, 603-4 (1941).

few metals that form stable lakes with the hydroxyanthraquinones. The lakes formed with alizarin, 1,2-dihydroxyanthraquinone, and purpurin, 1,2,4-trihydroxyanthraquinone, are red and dark red respectively, while the quinalizarin, 1,2,5,8-tetrahydroxyanthraquinone, lake is purple. Dithizone, or diphenylthiocarbazone, as the lake-forming reagent,[2] is even more sensitive than the hydroxyanthraquinones, but it is less stable and less easily prepared. Transmittance measurements will estimate as little as 0.01 mg. of the isolated metallic cation suspended in the lake form.[3]

Above 560 mμ for alizarin, 580 mμ for purpurin and 620 mμ for quinalizarin, the absorption due to the dye is negligible and Beer's law holds. These were found to be the optimum conditions. At longer wave lengths, the amount of light scattering by the particles of the dye lake increases and light absorption correspondingly decreases. Scattering is most marked in the case of purpurin and least noticeable in the case of alizarin. Increasing the acid concentration decreases the particle size and hence increases the amount of light transmitted. Sulfuric acid and hydrofluoric acid form stable zirconium complexes and therefore diminish the lake formation. This method is applicable to the indirect colorimetric determination of sulfate ion or fluoride ion.

Many cations, if present in largo excess, interfere either by forming lakes or zirconyl complexes; the former difficulty may be overcome by adding an excess of acid although this diminishes somewhat the sensitivity. The use of perchloric acid instead of hydrochloric acid minimizes the tendency of zirconium ions to form complexes. The presence of ferric iron, chromium, and cobalt depresses the transmittance curve; tungsten and molybdenum increases the steepness of the curves; cadmium, copper, lead, and aluminum in the presence of hydrochloric acid retard lake formation and increase the transmittance. Comparable amounts of these elements in sample and standard do not alter the results significantly. Thorium causes a small error; titanium introduces a greater source of interference. The absorption spectra of titanium and zirconium are closely related, thus making it more difficult to distinguish between them.

If all other metallic ions are removed from the solution, the proportions of a mixture of hafnium and zirconium salts, whose transmittance curves are very closely related, can be estimated, since one gram of zir-

[2] Herman A. Liebhafsky and Earl H. Winslow, *J. Am. Chem. Soc.* 59, 1966-71 (1937).

[3] Herman A. Liebhafsky and Earl H. Winslow, *ibid.* 60, 1776-84 (1938).

CHAPTER 24

VANADIUM

VANADIUM is an important alloying element, present in small amounts in igneous rocks and often associated with lead, copper, etc. The reagents usually applied to develop a color are hydrogen peroxide or phosphotungstic acid.

SAMPLES

Ferrous Alloys. *Vanadium Steel.* If the sample is a high-vanadium steel, containing from 0.7-2.0 per cent of the metal, weigh out a 0.15-gram sample. Add 10 ml. of 1:1 hydrochloric acid, cover, and warm until all action has ceased. Add a drop of 1:1 nitric acid and boil with continuous swirling. Continue to add nitric ocid dropwise until the tungsten is oxidized, or until tho residue is bright yellow with no black particles.

If the sample contains 0.1-0.7 per cent of vanadium, weigh out a 0.5-gram sample. Add 20 ml. of 1:1 hydrochloric acid, cover, and warm until all action has ceased. Add 0.5 ml. of concentrated nitric acid to oxidize the iron, evaporate to 10 ml., and add 5 ml. of concentrated hydrochloric acid. Transfer to a separatory funnel with not more than 5 ml. of water. Cool, add 30 ml. of ether, and extract most of the iron. Warm tho aqueous layer on a hot plate to remove the ether.

To either sample in acid solution, add 7 ml. of 60 per cent perchloric acid and evaporate to fumes. Boil gently until the solution assumes an orange-red dichromate color, then 2-3 minutes longer. Cool, add 40 ml. of water, heat to boiling, and add 5 ml. of 23 per cent lead perchlorate solution. Cool to room temperature and filter through a Gooch crucible. Wash with a little water and dilute to a known volume. This method lends itself to determination of vanadium by phosphotungstic acid.

Alternatively, weigh 0.2 gram if the vanadium is less than 0.5 per cent, or 0.1 gram if over that amount. Prepare a mixture of 1 part of 85 per cent orthophosphoric acid, 6 parts of concentrated sulfuric acid, and 33 parts of water. Add 3 ml. of this mixture. Samples containing chromium dissolve readily to a clear solution. Tungsten remains as a black residue. Add 1 ml. of 1:1 nitric acid and heat until no more nitric oxide fumes are given off. Cool and add 1 ml. of 10 per cent ammonium

persulfate solution. Heat until evolution of gas ceases. Cool and, if necessary, allow to stand to permit the phosphomolybdate to precipitate. Dilute to a known volume.

Tungsten, Chromium, Titanium and Molybdenum Steels. Weigh a 2-gram sample and dissolve in 40 ml. of 1:1 nitric acid. Filter precipitated tungstic acid. Render the filtrate just alkaline to litmus with 1:1 ammonium hydroxide and evaporate to dryness. Take up with 20 ml. of 1:1 nitric acid and filter the tungstic acid. Evaporate to about 10 ml. Pour into a separatory funnel and rinse the dish with 10 ml. of 1:1 hydrochloric acid in several portions. Add 50 ml. of alcohol-free ether and extract the bulk of the iron. Draw off the acid solution and a little ether with it. Titanium, vanadium, and chromium are in the acid layer. Molybdenum is in the ether layer with the iron.

Evaporate the acid solution nearly to dryness. Add 5 ml. of concentrated nitric acid and again evaporate nearly to dryness. This removes all chlorides. Add 20 ml. of concentrated nitric acid and heat to boiling. Add 2 grams of potassium chlorate and continue boiling for about 1 minute. Chromium and manganese are oxidized. Dilute to about 250 ml. Manganese goes into solution. Heat to boiling and slowly neutralize by addition of 1:1 ammonium hydroxide. Residual iron is precipitated and occludes the vanadium. Titanium is also precipitated, but chromium is in solution. Filter and wash with hot water.

Transfer the filter to a dish and dissolve the precipitate in a maximum of 9 ml. of 1:5 hydrochloric acid by heating. Filter to remove paper fibers and manganese, and wash, using a volumetric flask as receiver. Dilute to a known volume for the use of aliquots for determination by the peroxide method. It is necessary to add hydrofluoric acid in the procedure to take care of residual iron and titanium.

Iron Ores and Slags.[1] Transfer 1 gram of finely powdered sample to a nickel crucible and fuse with 8 grams of sodium peroxide. Plunge the hot crucible into cold water, add 50 ml. of water to the contents, and warm to disintegrate the melt. Remove the crucible and add 65 ml. of concentrated nitric acid to the water solution. Heat and add 2 ml. of 15 per cent hydrogen peroxide with stirring. No colored residue should remain. Continue to boil for 3-5 minutes to decompose pertitanate, vanadate, chromate, and excess hydrogen peroxide. Cool to 20°, transfer the green solution to a 250-ml. volumetric flask, and dilute to volume. Use half as the aliquot and the other half for the photometric blank.

[1] H. Pinsl, *Arch. Eisenhüttenw.* 11, 293 6 (1937).

Alternatively,[2] heat 1 gram of finely ground sample with 15 ml. of concentrated nitric acid and 45 ml. of concentrated hydrochloric acid. Evaporate to dryness on a sand bath. Cool and dissolve the residue in 25 ml. of concentrated nitric acid. Evaporate to about 5 ml., cool, and dilute to 25 ml. with hot water. Filter and evaporate the filtrate to dryness. Cool and dissolve in 20 ml. of 1:1 nitric acid. Add 2 ml. of 10 per cent silver nitrate solution and boil for several minutes to remove chlorides. Cool and filter. Add 10 ml. of 0.8 per cent potassium permanganate solution to the filtrate and boil to remove remaining organic matter. Add 30 per cent hydrogen peroxide to remove excess potassium permanganate and precipitated manganese dioxide. Boil to destroy excess peroxide. Cool and transfer to a 100-ml. volumetric flask. Dilute to volume and use aliquots.

Minerals. A solution was prepared for determination of chromium (page 270). Use 10-50 ml. as an aliquot for vanadium and proceed as follows.[3] Add 1 drop of methyl orange indicator solution to the aliquot and 1:8 sulfuric acid from a buret until the intermediate color of the indicator is reached. Swirl to liberate dissolved carbon dioxide and transfer to a separatory funnel. Add 2 ml. of chloroform and 0.1 ml. of a 2.5 per cent solution of 8-hydroxyquinoline in 1:8 acetic acid. Shake for 1 minute and let stand for the layers to separate. Vanadium gives a red to black color, probably of $(C_9H_6ON)_4 \cdot V_2O_3$. Iron gives a greenish black of equal sensitivity. Withdraw the chloroform layer, which now contains much of the vanadium, iron, uranium, and a trace of molybdenum, into a platinum crucible. Repeat the addition of reagent and chloroform twice more. The final extract should show only a faint yellow of the 8-hydroxyquinoline.

Add 0.1 gram of sodium carbonate to the chloroform extracts and evaporate to dryness. Heat the crucible with a free flame to destroy organic matter and finally apply the full flame to fuse as sodium vanadate. Warm the cooled residue with 3-4 ml. of water to dissolve and use as sample for development by phosphotungstic acid. Rinse directly into the tube to be used for development of color.

As an alternative, treat a finely powdered 1-gram sample with 2 ml. of 48 per cent hydrofluoric acid and 6 drops of concentrated sulfuric acid. Evaporate to dryness. Add 5 grams of sodium acid sulfate and fuse. Cool and dissolve in 25 ml. of 1:10 sulfuric acid. Filter, if neces-

[2] K. Bolshakov, *Tzvetnuie Metal.* 6, 487-93 (1931).

[3] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 8, 336-41 (1936).

sary, and transfer to a 50-ml. volumetric flask. Add 1 ml. of 85 per cent orthophosphoric acid and dilute to volume. Use an aliquot as sample.

Plant Ash. Dissolve the residue from dry ashing 2.0 grams of sample in 10 ml. of the concentrated nitric acid and dilute to about 50 ml. Filter and wash the filter with hot water until free from acid. Precipitate chlorides from the sample solution with 1 ml. of 10 per cent silver nitrate solution and filter. Oxidize the filtrate with 0.8 per cent potassium permanganate solution until destruction of any adventitious organic matter is complete. Remove manganese dioxide and excess of potassium permanganate by addition of 30 per cent hydrogen peroxide. Boil to destroy the excess of peroxide. Transfer to a 100-ml. volumetric flask, dilute to volume, and use an aliquot.

Standards

If ammonium vanadate of high purity is available, dissolve 0.2295 gram in about 100 ml. of water with addition of 15 ml. of 1:1 nitric acid and dilute to a liter. This will contain 0.1 mg. of vanadium per ml.

Otherwise,[4] dissolve vanadium pentoxide in a slight excess of sodium hydroxide, filter the solution, and add ammonium chloride solution to precipitate ammonium vanadate. Filter, dry, and ignite in a muffle at 500° to purified vanadium pentoxide. Dissolve 1.819 grams of the pentoxide in excess of sodium hydroxide solution, make slightly acid with concentrated sulfuric acid, cool, and dilute to 1 liter. To standardize, take 2 portions of 200 ml. Add to each 25 ml. of 1:1 sulfuric acid and heat to boiling. Add 30 ml. of aqueous sulfurous acid. Boil off excess sulfurous acid and titrate hot with a standard potassium permanganate solution. The ml. of permanganate times the normality times 0.051 gives the amount of vanadium present. Add 50 ml. of dilute nitric acid to the remaining solution and dilute until 1 ml. corresponds to 0.1 mg. of vanadium.

Vanadium by Hydrogen Peroxide

Vanadium is determined by the reddish brown color produced when quinquevalent vanadium and hydrogen peroxide react in acid solution. So long as mineral acid is used, the particular acid selected makes little difference. The color is either due to peroxyvanadic acid, HVO_4, or to a complex peroxidized sulfate. Comparatively little peroxide is required

[4] E. R. Wright and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 9, 251-4 (1937).

to develop the maximum color, whereas a large excess causes a diminution in color intensity. The color is stable for at least 2 days. The optimum concentration of hydrogen peroxide appears to lie at 0.2-3 ml. of 3 per cent solution per 100 ml. Maximum absorption occurs at 460 mμ.[5] The optimum acid concentration is above 0.6 *N*. No change then occurs to 6 *N*. The system conforms to Beer's law.[6]

The presence of 0.05 mg. of titanium in the sample is sufficient to cause a 2 per cent deviation in the vanadium determination because of reaction with the reagent. Molybdenum and tungsten react similarly but interfere less. Chromium as chromate gives a similar color. Interference, except by titanium, may be overcome with moderate accuracy by using a standard containing the same interfering substances as the sample. The addition of orthophosphoric acid results in the formation of an insoluble tungsten compound. Either orthophosphoric acid or fluoride prevents interference by iron. Iodide and bromide must be absent. Interference from combined carbon is eliminated by treatment with persulfate or permanganate.

Corrections can be applied for titanium, molybdenum,[7] and other alloying metals, with the exception of tungsten which must be removed.[8] Ammonium persulfate may be used to oxidize any chromium present. Hydrofluoric acid [9] or sodium fluoride prevents interference by decolorizing titanium quantitatively.

Vanadium is also determined by hydrogen peroxide, in a simultaneous determination with titanium, using two different bands, one centering at 430 or 436 mμ and the other at 546 or 565 mμ. Calibration curves can be prepared for each band, plotting the extinction coefficients against percentage of titanium and vanadium,[10] but it is more convenient to calculate the results.

Vanadium, titanium, and molybdenum are determined with fair accuracy by monochromatic light at wave lengths corresponding to the absorption peaks of the three complexes.[11] Perchloric acid is added to the solution, and the transmittance of a peroxidized aliquot is measured against an unperoxidized aliquot. If orthophosphoric acid is used to eliminate ferric-ion color, it narrows the absorption band around 400 mμ.

[5] E. R. Wright and M. G. Mellon, *ibid.* 9, 375-6 (1937).
[6] Margaret D. Foster, *U. S. Geol. Survey Bull.* No. 950, 7-13 (1946).
[7] F. W. Haywood and A. A. R. Wood, "Metallurgical Analysis by Means of the Spekker Absorptiometer," pp. 71-3. Adam Hilger & Sons, London, England (1943).
[8] H. Pinsl, *Giesserei* 27, 441-6 (1940).
[9] Louis Silverman, *Ind. Eng. Chem., Anal Ed.* 14, 791-2 (1942).
[10] H. Pinsl, *Angew. Chem.* 50, 115-20 (1937).
[11] Alfred Weissler, *Ind. Eng. Chem., Anal. Ed.* 17, 695-8 (1945).

Procedure. Measure out an aliquot of sample to contain 1-5 mg. of vanadium and dilute to about 80 ml. Allowing for the acid already present, add sufficient 1:1 sulfuric acid to give a total of 3-6 ml. of concentrated acid present. If the sample contains titanium add 1 ml. of 48 per cent hydrofluoric acid to prevent development of color from it. This will also decolorize iron. If iron is present but no titanium, substitute 2 ml. of 85 per cent orthophosphoric acid. Add 3 ml. of 3 per cent hydrogen peroxide and dilute to volume. If hydrofluoric acid is used in the sample, compare with a series of standards. Otherwise read photometrically against a similarly prepared sample to which 3 ml. of water has been added in place of the hydrogen peroxide.

Vanadium and Titanium. The method is given under titanium (page 441).

Vanadium, Titanium, and Molybdenum. The method is given under titanium (page 442).

Vanadium by Phosphotungstic Acid

A solution of quinquevalent vanadium treated with sodium tungstate and orthophosphoric acid yields an immediate, stable yellow or brownish yellow phosphotungstovanadic acid, varying in hue and intensity with the concentration of vanadium ions present.[12] Vanadate gives a color with tungstate alone in slightly acid solution but the color is greatly intensified when orthophosphoric acid is present. The reaction is several times as sensitive as that with hydrogen peroxide, and the color is more easily matched.

The molecular ratio of sodium tungstate to orthophosphoric acid may vary from 3 to 20 without causing any change visible to the naked eye. However, the concentration of sodium tungstate should be limited to the range of 4-34 grams per liter. The acid concentration of the solution is not critical[13] If molybdenum is present in concentrations exceeding 50 mg. per 100 ml., interference occurs because of the formation of a color with the phosphotungstic acid. Tin, titanium, zirconium, and ammonium ions interfere. Furthermore, the presence of over 1 mg. of chromium as chromate, 10 mg. of cobalt or copper, 35 mg. of nickel,

[12] A. P. Vinogradov, *Compt. rend. acad. sci.* (U.S.S.R.) 1931A, 249-52; E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 8, 336-41 (1936); E. R. Wright and M. G. Mellon, *ibid.* 9, 251-4 (1937).

[13] G. A. Panchenko and R. I. Marder, *Trudy Tsentral. Lab. Zavoda "Bol'shevik"* 1940, 203 8; *Khim. Referat. Zhur.* 4, 75 (1941).

40 mg. of manganese, or 100 mg. of uranium or iron per 100 ml. serves as interference as a result of the color of the ions themselves.

The slightly acidified solution of vanadium may also be extracted with chloroform after the addition of an acetic acid solution of 8-hydroxyquinoline as described for minerals (page 452). Evaporation, ashing, and fusion of the residue with sodium carbonate follow. When the color is developed, accurate results within the range of 0.001-0.1 per cent vanadium oxide are obtained. The maximum absorption occurs at 440 mμ. The color is stable for a period of at least 2 days. As little as 0.002 mg. of vanadium can be determined.

Procedure. Transfer a sample containing 0.02-0.2 mg. of vanadium to a Nessler tube, and a slightly lesser volume of water containing the same reagents to another similar tube. To each add successively 1 ml. of 1:8 sulfuric acid, 0.3 ml. of 85 per cent orthophosphoric acid, and 0.2 ml. of 5 per cent sodium tungstate solution. Add a suitable vanadium standard to the duplicate until the tubes match, adjusting the volumes as usual.

The color may also be read photometrically at 400 mμ. Set the 100 value of the photometer with the undeveloped sample solution rather than water, so as to correct for any colored ions present.

Miscellaneous

A small amount of stannous chloride will reduce the complex formed on adding tungstic acid to an orthophosphoric acid solution of sample containing vanadium,[14] to give the usual molybdenum blue. The absolute error is not more than 0.5 per cent.[15] For determination add 3 ml. of 85 per cent orthophosphoric acid to 10 ml. of sample solution and dilute to about 15 ml. Heat to boiling and add 4 ml. of 5 per cent tungstic acid solution. Heat to about 95°, cool, and add 1 ml. of 0.5 per cent stannous chloride in 1:10 hydrochloric acid. Dilute to 25 ml. and read the color photometrically or by balancing against a standard.

This method is modified in the treatment of sulfuric-phosphoric acid solutions with nitric acid to oxidize the vanadium. Then addition of ammonium molybdate results in the formation of a yellow-orange phosphovanadomolybdate complex. Examination is made under mercury

[14] A. L. Davydov and Z. M. Vaisberg, *Zavodskaya Lab.* 9, 715-23 (1940); V. A. Romashchenko *ibid.* 11, No. 1, 104 (1945).

[15] E. I. Grenberg and M. Ya. Genis, *ibid.* 9, 1145-6 (1940).

light, as the maximum absorption occurs at 436 mμ.[16] Chromium need not be oxidized to chromic acid, but subtract 0.015 per cent of vanadium for each 1 per cent of chromium, and 0.01 per cent of vanadium for each 10 per cent of cobalt. As much as 10 per cent of molybdenum and titanium and 20 per cent of tungsten may be present without interfering. The method is applicable in the range 0.01-4.6 per cent.

Treatment of a neutral solution of ammonium vanadate with a neutralized solution of ammonium molybdate results in a yellow solution,[17] which is stable for several hours. In acid solution the color fades completely in 5 minutes. For the determination, add 10 ml. of freshly prepared 10 per cent ammonium molybdate solution to the sample solution in a Nessler tube and dilute to 100 ml. Compare with a standard of comparable composition similarly treated. By this means it is possible to detect 0.002 mg. of vanadium pentoxide in 100 ml. of solution.

Vanadium in water solution is determined with a sensitivity of 0.08 ppm. by treating with 8-hydroxyquinoline and extracting the violet color with isoamyl alcohol. The coloration follows Beer's law for concentrations of 0.1-0.5 mg. of vanadium per liter.[18] Iron, copper, and titanium interfere. Iron can be removed by precipitation with ammonium hydroxide and titanium inactivated by fluorides. For the determination acidify an aliquot of sample, containing 0.01-0.1 mg. of vanadium, to Congo red with sulfuric acid and dilute to about 50 ml. Add 0.2 ml. of glacial acetic acid and 5 drops of 2.5 per cent solution of 8-hydroxyquinoline in 1:9 acetic acid solution. Shake with 10 ml. of amyl alcohol to extract the color and compare the amyl alcohol extract with a standard similarly prepared.

8-Hydroxyquinoline-5-sulfonic acid and its halogen derivatives[19] form a permanent brown color used in the estimation of metavanadates with 2 per cent accuracy for solutions containing around 2.6 mg. of vanadium per liter. The error in more concentrated solutions is less. Iron gives a like reaction.

When a vanadic acid solution is reduced with aniline and hydrochloric acid, the blue compound that forms is the basis of a rapid colorimetric method for the determination of vanadium.[20]

[16] Gerold Bogatzki, *Arch. Eisenhüttenw.* 12, 539-42 (1939).

[17] A. G. Woodman and L. L. Cayvan, *J. Am. Chem. Soc.* 23, 105 (1901).

[18] José M. Bach and Rogelio A. Trelles, *Anales soc. quím. Argentina* 28, 111-22; *Bol. obras sanit. nación* (Buenos Aires) 4, 135-9 (1940); 5, 127-8 (1941).

[19] Jacob Molland, *Tids. Kjemi Bergvesen* 19, 119-22 (1939); *Compt. rend.* 210, 144-6 (1940).

[20] V. A. Zilbermintz and K. P. Florenzkii, *Mikrochemie* 18, 154-8 (1935).

An aqueous solution of diphenylamine produces a violet color with vanadium, detecting 2.5 ppm.[21] The optimum temperature for the reaction is 50°. Titanium and small amounts of iron and nitrates do not interfere. Active oxidizing agents must be absent. The reaction is much more sensitive than that with peroxide.

As reagent, heat 0.2 g. of diphenylamine with 100 ml. of water on a water bath, cool and filter. This solution is not affected by exposure to air or light. For the determination, add 5 ml. of concentrated hydrochloric acid and 5 ml. of reagent to a 50 ml. sample and to a similar standard. A violet color will appear within 2 minutes. Compare after 10 minutes by dilution. The same reaction is given by diphenylamine sulfonic acid.

Benzidine in phosphoric acid solution is oxidized to an intense yellow color by vanadium, a reaction which will detect 0.2 ppm.[22] Interference by chromium and manganese is avoided by addition of ferrous ammonium sulfate and sodium nitrite.

Even in the presence of 6 times as much chromium, vanadium may be determined by means of a cupferron reagent.[23] The color formed is colloidal, and iron, titanium, and large amounts of fluorides must be absent. The reagent will detect 1 ppm. Potassium sulfate is added to the sulfuric acid solution of the vanadium salt, a clear gum arabic solution is introduced as stabilizer, then cupferron is added, and comparison is made colorimetrically or nephelometrically against a similar standard.

A violet color, followed by an intense orange, occurs when a concentrated sulfuric-acid solution of strychnine is added to a vanadium solution [24] in small volume. Titanium, molybdenum, and tungsten do not interfere, but iron must be removed. For development of color, add 20 ml. of a 0.4 per cent solution of pure strychnine in concentrated sulfuric acid to 1 ml. of sample and standard. After 10 minutes compare the two by diluting the darker with concentrated sulfuric acid. The color is permanent for several hours.

When sodium sulfide is added to an alkaline vanadate solution, the resulting red thiovanadate is suitable for colorimetric estimation.[25] Vanadate forms a red complex with diphenylcarbazone in acetone acidi-

[21] Victor L. Meaurio, *Anales soc. quim. Argentina* 5, 185-9 (1917); *Ann. chim. anal.* 23, 47-50 (1918).

[22] I. P. Alimarin, *J. Applied Chem.* (U.S.S.R.) 17, 83-93 (1944).

[23] D. N. Finkel'shteĭn and L. P. Elenevich, *Zavodskaya Lab.* 7, 665-70 (1938).

[24] A. W. Gregory, *Chem. New* 100, 221 (1909).

[25] E. Stengel, *Tech. Mitt. Krupp Forsch. Ber.* 2, 93-4 (1939).

fied with acetic acid.[26] Titanium does not interfere and moderate amounts of chromate are tolerated. Molybdate and tungstate must be absent.

An approximation of vanadium content is obtained from the color of the melt of a volumetrically measured microsample of finely ground rock fused with potassium pyrosulfate.[27] With little interference from other elements 0.001 per cent of vanadium is detected. Nickel, manganese, uranium, and some rare earths may be mistaken for vanadium.

[26] Fritz Feigl and F. L. Lederer, *Monatsh.* 45, 63-8, 115-32 (1924); P. Krumholz and F. Hönel, *Microchim. Acta* 2, 177-83 (1937).

[27] Joseph M. Axelrod, *U. S. Geol. Survey Bull.* No. 950, 19-23 (1946).

acid and 40 ml. of concentrated hydrochloric acid to 0.5-2 grams of finely ground sample. Cover, digest on a steam bath for 20 minutes, then evaporate to dryness. Cool, add 10 ml. of 1:4 hydrochloric acid, and digest for 10-15 minutes. Cool, dilute to 50 ml., and warm. Add a small ball of paper pulp, filter into a 100-ml. volumetric flask, and wash the residue with water. Dilute the combined filtrate and washings to volume and use 10 ml. as aliquot by the thiocyanate method.

Molybdenum and Vanadium Absent. Heat 0.1 gram of sample with 1 ml. of 48 per cent hydrofluoric acid and 2 drops of 1:1 sulfuric acid. Add 2 drops more of sulfuric acid and heat again. Allow to cool, and fuse the residue with 0.5 gram of sodium carbonate. Cool and dissolve in hot water. Add a few drops of ethanol to reduce manganates, and filter. Wash the filter and evaporate the combined filtrate and washings to dryness. Dissolve the salts in 2 ml. of water and use as sample.

Low Tungsten Content. When less than 0.02 per cent of tungsten is present, proceed as in the previous paragraph through "Cool and dissolve in hot water." Dilute to about 100 ml. and acidify to methyl orange with 1:4 hydrochloric acid. Add about 0.03 gram of ferric chloride and render the solution faintly alkaline with 1:1 ammonium hydroxide to precipitate the iron and carry the tungsten down with it. Filter and ash. Resume according to the preceding method, starting at "Allow to cool and fuse the residue with 0.5 gram of sodium carbonate."

Tin-tungsten-arsenic Ores.[5] Treat 0.2-1.0 gram of 250-mesh ore in a crucible with 30 ml. of concentrated hydrochloric acid. Warm to 60° on a hot plate and keep at that temperature for an hour. Cool, add 5 ml. of concentrated nitric acid, and evaporate to dryness. Moisten the dry residue 3 times with concentrated hydrochloric acid and, in each case, evaporate to dryness. Cool, add 30 ml. of concentrated hydrochloric acid and 0.5 gram of hydrazine hydrochloride or sulfate, and evaporate to 5 ml. Add 30 ml. of concentrated hydrochloric acid and evaporate to dryness. Arsenic will have been volatilized as the chloride. Cool, add 5 ml. of 20 per cent sodium carbonate solution, and heat gently for 30 minutes. To the solution, add dropwise 3 per cent hydrogen peroxide until a brown color appears. Filter and use as sample, or dilute to 100 ml. and use aliquots, applying the thiocyanate method.

[5] P. V. Faleev, *Zavodskaya Lab.* 8, 1174-5 (1939).

Sulfide Ores.[6] Roast a 0.5-gram sample in an iron crucible at not over 850° until the sulfur has been completely driven off as oxides. Fuse the cooled sample with 2 grams of sodium hydroxide. Take up with about 50 ml. of water and filter into a 100-ml. volumetric flask. Dilute to volume for the use of aliquots by the thiocyanate method.

Concentrates.[7] Melt a 4-gram portion of sodium hydroxide in an iron crucible. Add a 0.25-gram sample of concentrate or 0.5 gram of rich ore and fuse for 15-20 minutes. Cool and dissolve in 50 ml. of hot water. If the solution is green or blue, add 3 drops of ethanol and 2 drops of 37 per cent solution of formaldehyde. Boil until colorless and the residue is well disintegrated. Transfer to a 100-ml. volumetric flask, dilute to volume, mix, and let settle. Transfer a suitable aliquot of the sample solution to a 100-ml. volumetric flask and dilute to volume with 1.5 per cent sodium hydroxide solution. Use an aliquot of this as sample for reduction by zinc in the presence of thiocyanate.

Silicate Minerals.[8] To a 1-gram sample in platinum add 5 ml. of 1:5 sulfuric acid, 2 ml. of concentrated nitric acid, and 5 ml. of 48 per cent hydrofluoric acid. Evaporate to dryness and heat so long as fumes are given off. To the cooled residue add 1 ml. of 1:5 sulfuric acid and 5 ml. of water. Warm and stir until dissolved as much as possible and evaporate to dryness. Heat to drive off fumes of sulfuric acid. Add 1 ml. of 1:5 sulfuric acid and 10 ml. of water. Digest just below boiling for 30 minutes with occasional stirring. There may be undissolved residue in suspension.

Heat 15 ml. of 10 per cent sodium hydroxide solution nearly to boiling and add the solution of the mineral sample dropwise while stirring. Pour this into the platinum dish and digest just below boiling for 15 minutes. Filter through a sintered glass crucible, collecting the filtrate in a tube inside the suction flask. Wash the precipitate with 2-3 ml. of water. Transfer the filtrate to a beaker and place on a steam bath to evaporate.

Transfer the main portion of the precipitate to the beaker in which precipitation was carried out. Rinse the platinum dish with 5 ml. of 1:1 hydrochloric acid and pour this into the crucible without suction. After the residual precipitate in the crucible has dissolved, pour into the beaker containing the precipitate. Wash the crucible with a few ml.

[6] A. T. Voznesenskii, *ibid.* 9, No. 1, 25-8 (1940).
[7] O. A. Songina and P. A. Karpova, *ibid.* 13, 38-42 (1947).
[8] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 18, 163-7 (1946).

Procedure. Use a solution of sample in sulfuric and orthophosphoric acids and evaporate to fumes. Cool and add 5 ml. of 1:2 hydrochloric acid. Add 5 drops of 10 per cent solution of hydrazine sulfate and 10 ml. of a 1 per cent solution of toluene-3,4-dithiol in amyl acetate. Shake occasionally for 15 minutes, then separate. Discard the extract and heat the aqueous layer to drive off solvent. Then add a few drops of concentrated nitric acid and evaporate to fnmes. Take up in 2 ml. of concentrated nitric acid, add 2 ml. of water, and again evaporate to fumes. Take up in 5 ml. of a 10 per cent solution of stannous chloride in concentrated hydrochloric acid by heating for 5 minutes on a water bath. Add 10 ml. of 1 per cent dithiol solution in amyl acetate and continue to heat for 10 minutes, shaking occasionally. Transfer to a separatory funnel with three 2-ml. portions of amyl acetate. Shake and discard the aqueous layer. Wash the solvent layer with 5 ml. of concentrated hydrochloric acid and discard these washings. Dilute to a known volume with amyl acetate as a developed sample for reading the transmittance.

Tungsten by Hydroquinone

If hydroquinone is added to tungstic acid in concentrated sulfuric acid solution, a red color forms.[22] The presence of moisture can be corrected by adjusting the concentration of hydroquinone. Thus roughly 1 gram of hydroquinone per 100 ml. will correct for 1 gram of water in the same volume and maintain a good, clear color. The system conforms to Beer's law. Nitric acid must be absent; hydrochloric acid modifies the color. The minimnm transmittance is around 465 mμ, but errors due to contaminants are greater at that level. Thus the color is preferably read at 570 mμ. Ferric and molyhdate ions are reduced with stannous chloride to remove interference. Titanium, columbium, and perrhenate ions interfere. A correction factor, corresponding to 0.01 per cent of tungsten for each per cent of chromium, may he subtracted. The method is applicable to 0.2-20 per cent of tungsten and has an accuracy of 10-20 per cent.

Procedure. Prepare a reagent to contain 10 grams of hydroquinone in 100 ml. of concentrated sulfnric acid. First test the aqueous sample solution hy mixing equal volumes of it and this reagent. If a red color is ohtained under those conditions, titaninm present prevents the use

[22] G. Heyne, *Z. angew. Chem.* **44**, 237-8 (1931); G. Bogatzki, *Z. anal chem.* **114**, 170-81 (1938); Charles M. Johnson, *Iron Age* **157**, 66-9 (1946).

of this method. The sample solution should have been reduced with stannous chloride in acid solution. To 1 volume of sample solution add 9 volumes of reagent. Read the transmittance with a filter centering around 570 mμ.

For samples free from iron, titanium, and molybdenum, greater sensitivity is obtainable as follows. Neutralize the sample solution and add 0.5 ml. of a 10 per cent solution of potassium hydroxide. Evaporate to dryness and cool. Add 0.5 ml. of concentrated sulfuric acid and heat until sulfur trioxide fumes are evolved. Cool and add 1 ml. of the hydroquinone reagent. Dilute to a suitable volume with concentrated sulfuric acid and compare with a standard similarly treated.

Miscellaneous

The addition of Rhodamine B to tungsten in dilute hydrochloric acid solution converts the yellowish red color of the dye to a reddish violet of a colloidal dispersion.[23] The color does not conform to Beer's law. The method is applicable to samples containing less than 10 times as much molybdenum, and its accuracy is only 20-30 per cent. Excess hydrochloric acid interferes, but sodium chloride and silicates do not. The color is stable for only a few minutes on heating but for several day in the cold. As a typical procedure, evaporate an acid sample to a small volume to free it from volatile acids. Neutralize and evaporate to 0.5 ml. Add 1 drop of concentrated hydrochloric acid and 2 ml. of a solution containing 0.1 gram of Rhodamine B per liter. Compare by both reflected and transmitted light with standards similarly treated at the same time.

Tungstic acid in dilute hydrochloric acid may be reduced with titanium trichloride[24] or stannous chloride to give a blue oxide which remains in colloidal suspension for 30 minutes. More than 0.1 N hydrochloric acid lessens the color and it fades completely at 0.5 N. Vanadium and phosphorus interfere, and the presence of molybdenum modifies the color and renders it unstable. The method is accurate to 2 per cent, but the amount of tungsten in the final solution must be limited to 1 mg. per ml to prevent coagulation of the oxide. Reduction may also be carried out using powdered lead.[25] A suitable reagent is a solution of titanium trichloride corresponding to 2 mg. of iron per ml. To a convenient volume of sample, adjusted to about 0.05 N with hydrochloric

[23] G. Heyne, *Z. angew. Chem.* **44**, 237-8 (1931).
[24] A. Travers, *Compt. rend.* **165**, 408-10 (1917); *ibid.* 166, 416 (1918).
[25] A. Petrovskii, *J. Chem. Ind.* (Moscow) **7**, 905-7 (1930).

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.. remove interference. Titanium, columbium, and
interfere. A correction factor, corresponding to 0.01
...ten for each per cent of chromium, may be subtracted.
is applicable to 0.2-20 per cent of tungsten and has an
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... Prepare a reagent to contain 10 grams of hydroquinone
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under those conditions, titanium present prevents the use

Z. angew. Chem. 44, 237-8 (1931); G. Bogatzki, *Z. anal chem.* 114,
... M. Johnson, *Iron Age* 157, 66-9 (1946).

CHAPTER 26

MOLYBDENUM

Molybdenum is widely distributed as molydates. In recent years the mount of molybdenum in soils and plants has been found to be a critical ıctor. The metal is an important steel-alloying element. With the ıcrease in applications, and appreciation of the importance of the metal, as come a demand for rapid methods of analysis for what often proves ɔ be minute quantities. Colorimetric determination of the thiocyanate omplex of molybenum is generally employed, although several other ıethods have been used successfully.

SAMPLES

Tungsten and Molybdenum Wire. To a 4-gram sample in a platinum crucible add 30-40 ml. of 48 per cent hydrofluoric acid. Add 1:1 itric acid dropwise, according to the violence of the reaction, until olution is complete. Evaporate nearly to dryness. Add 5 ml. of conntrated nitric acid and evaporate to dryness. Repeat this 3 times. gnite at not over 550° to eliminate any organic matter and to break p tungstic acid hydrates and complexes.

Dissolve in 2 ml. of 10 per cent sodium hydroxide solution. No residue f tungsten should remain. Filter, if necessary, on an inorganic filter. Vash the residue with 5 per cent sodium chloride solution. Dilute to 50 ml. and take a 50-ml. aliquot for determination by the thiocyanate ethod.

Tungstic Acid.[1] Treat a 5-gram sample with 5 ml. of concentrated itric acid and proceed as for wire, starting at "Evaporate nearly to ryness"

Steel. For steel and iron[2] containing no more than 0.25 per cent molybdenum use 0.2 gram of sample; for 0.25-0.6 per cent use 0.1 ram; for 0.6-1.4 per cent use 0.05 gram. When large percentages of

[1] Walter J. King, *Ind. Eng. Chem.* 15, 350-4 (1923).

[2] George M. Poole, *Iron Age* 148, No. 15, 62, 164-5 (1941); Arba Thomas, *Proc. m Soc. Testing Materials* 44, 769-78 (1944).

acid, add a small excess of reagent over the amount necessary to give a maximum color. Compare with a standard solution of similar concentration prepared in the same way.

Alternatively, use a reagent consisting of 10.3 grams of stannous chloride and 2.1 grams of stannic chloride dissolved in 100 ml. of 10 per cent acetic acid and 40 ml. of 85 per cent orthophosphoric acid. A precipitate may form which should be well dispersed before use. To 40 ml. of sample solution and an equivalent standard, add 6 ml. of the reagent and concentrated sulfuric acid to make 50 ml. Compare after 5 minutes.

The addition of copper sulfate to a hot solution of tungstate results on cooling in the quantitative precipitation of copper tungstate. This may be filtered, washed with 80 per cent ethanol, dissolved in concentrated hydrochloric acid, and compared with standard copper solutions.[26]

[26] F. M. Shemyakin, A. V. Veselova and M. I. Vladimirova, *Zavodskaya Lab.* 5, 231-2 (1936).

CHAPTER 26

MOLYBDENUM

Molybdenum is widely distributed as molydates. In recent years the amount of molybdenum in soils and plants has been found to be a critical factor. The metal is an important steel-alloying element. With the increase in applications, and appreciation of the importance of the metal, has come a demand for rapid methods of analysis for what often proves to be minute quantities. Colorimetric determination of the thiocyanate complex of molybenum is generally employed, although several other methods have been used successfully.

SAMPLES

Tungsten and Molybdenum Wire. To a 4-gram sample in a platinum crucible add 30-40 ml. of 48 per cent hydrofluoric acid. Add 1:1 nitric acid dropwise, according to the violence of the reaction, until solution is complete. Evaporate nearly to dryness. Add 5 ml. of concentrated nitric acid and evaporate to dryness. Repeat this 3 times. Ignite at not over 550° to eliminate any organic matter and to break up tungstic acid hydrates and complexes.

Dissolve in 2 ml. of 10 per cent sodium hydroxide solution. No residue of tungsten should remain. Filter, if necessary, on an inorganic filter. Wash the residue with 5 per cent sodium chloride solution. Dilute to 250 ml. and take a 50-ml. aliquot for determination by the thiocyanate method.

Tungstic Acid.[1] Treat a 5-gram sample with 5 ml. of concentrated nitric acid and proceed as for wire, starting at "Evaporate nearly to dryness."

Steel. For steel and iron[2] containing no more than 0.25 per cent of molybdenum use 0.2 gram of sample; for 0.25-0.6 per cent use 0.1 gram; for 0.6-1.4 per cent use 0.05 gram. When large percentages of

[1] Walter J. King, *Ind. Eng. Chem.* 15, 350-4 (1923).

[2] George M. Poole, *Iron Age* 148, No. 15, 62, 164-5 (1941); Arba Thomas, *Proc. Am. Soc. Testing Materials* 44, 769-78 (1944).

tungsten are present, add 5-10 ml. of 85 per cent orthophosphoric acid. For 1.4-7 per cent of molybdenum with tungsten use 0.1 gram of sample and add 5-10 ml. of 85 per cent orthophosphoric acid. For higher concentrations of molybdenum, modify the sample downward. Above 1.4 per cent of molybdenum, aliquot as described at the end.

Add 20 ml. of 60 per cent perchloric acid per gram of sample and warm until disintegration is complete. A few drops of 48 per cent hydrofluoric acid will often expedite solution. Heat to boiling until all carbonaceous matter has disappeared. Cool somewhat, add 25 ml. of water, and boil until free chlorine is expelled. Add 2 grams of tartaric acid to prevent interference by tungsten or vanadium and a slight excess of 10 per cent sodium hydroxide solution. Heat at just under boiling for a few minutes. Remove from the heat, neutralize with 1:1 sulfuric acid, and add 1 ml. of the acid for each 4 ml. of solution to give 1:10 sulfuric acid. Cool and use this as sample by the thiocyanate method. For the lower concentrations of molybdenum it is not satisfactory to aliquot. For concentrations above 1.4 per cent, aliquoting is necessary, and reduction of the amount of stannous chloride in the procedure is advisable.

If sufficient chromium is present so that it should be eliminated, let cool, add 0.2 gram of sodium chloride to the strongly acid solution, and heat again until the orange color disappears and the sample again fumes strongly. Chromium will have been volatilized as the chloride. Let cool and wash the sample into a container for determination. At this stage it is suitable for the ASTM method if transferred with distilled water and diluted to 50 ml. The retention of any nitric acid will tend to deepen the color.[3]

If it is desired to determine molybdenum in iron by a method which does not permit the presence of iron in the final solution,[4] dissolve a 0.5 gram sample in 6 ml. of 85 per cent orthophosphoric acid and 35 ml. of 1:6 sulfuric acid. Add 1:1 nitric acid dropwise until the color of the boiling solution reaches a maximum, showing that oxidation is complete. Evaporate to sulfur trioxide fumes, let cool, and take up in water.

If tungsten need be removed, modify this by dissolving in 50 ml. of concentrated hydrochloric acid and evaporate to a red mass of crystals. Take up in 10 ml. of concentrated hydrochloric acid and oxidize by dropwise addition of concentrated nitric acid until a maximum color is reached. Add 1 ml. of 48 per cent hydrofluoric acid to decompose

[3] George M. Poole, *Iron Age* 148, No. 15, 62, 164-5 (1941).

[4] E. I. Fogel'son and F. S. Kazachkova, *Zavodskaya Lab.* 9, 783-4 (1940); R. Ladisch, *Chem.-Ztg.* 68, 27-8 (1944).

silica. Introduce a small amount of paper pulp, filter, and wash the paper free of iron. To the filtrate add 20 ml. of water and 4.5 ml. each of concentrated sulfuric acid and 85 per cent orthophosphoric acid. Bring to approximate neutrality with 10 per cent sodium hydroxide solution. Add 2 grams of ferrous sulfate and dissolve by stirring. This precipitates vanadium. Heat 100 ml. of 10 per cent sodium hydroxide solution to boiling and, while boiling vigorously, slowly pour in the sample solution with stirring. Cool and dilute to 500 ml. in a volumetric flask. When sedimented, pipet out a suitable aliquot of the upper layer, bearing in mind that it must be neutralized with 1:1 sulfuric acid before it is suitable for application as sample.

A solution was set aside in determination of copper (page 84) and is suitable for this determination.

Low-alloy Steel.[5] Prepare an acid mixture of 8 ml. of water, 10 ml. of 70 per cent perchloric acid, and 4 ml. of concentrated nitric acid. Heat a 0.075-gram sample with 1.5 ml. of this acid mixture until dissolved, and fumes of perchloric acid are driven off. Take up in 3 ml. of water and boil to drive off chlorine. Add 4.5 ml. of a mixture of 14.5 ml. of water, 4.5 ml. of concentrated sulfuric acid, and 1 ml. of concentrated hydrochloric acid. Heat until dissolved and use the method with thiocyanate and stannous chloride.

Nickel-chrome Alloys. Amounts from 0.1-7.0 per cent of molybdenum may be determined in steel and alloy steel containing nickel, chromium, vanadium, and tungsten with an accuracy of ±1-2 per cent,[6] as suggested in Table 6. The orthophosphoric acid added to dissolve tungsten serves to prevent precipitation of tungstic oxide, which would cause occlusion of molybdenum, and to stabilize the color.[7] The butyl acetate need not be saturated with reagents before extraction.

A solution was prepared for determination of chromium (page 267) of which an aliquot may be used for molybdenum.

Ferromolybdenum. Treat 1 gram of finely powdered ferromolybdenum in a porcelain dish with 10 ml. of 1:1 nitric acid. Decant the nitric acid solution. Fuse the insoluble residue with 2 grams of a mixture of sodium and potassium carbonates. Dissolve in 25 ml. of water and add to the nitric acid solution. Heat to the complete removal of nitric

[5] Rolland I. Mays, *Chemist-Analyst* 35 62-3 (1946).
[6] George M. Poole, *Iron Age* 148, No. 15, 62, 164-5 (1941).
[7] G. V. L. N. Murty, *Metallurgia* 35, 167-8 (1947).

TABLE 6. DETERMINATION OF SAMPLES WITH VARYING MOLYBDENUM CONTENT

Molybdenum Content	*Grams of Sample*	*Dissolve Sample in*	*Add*	*Add Stannous Chloride 112 Grams + 100 Ml. of Concentrated Hydrochloric Acid*	*Add Butyl Acetate*
0.01-0.60	0.1	20 ml. of 70% perchloric acid 10 ml. of water	50 ml. of water 10 ml. of 5% sodium thiocyanate	25 ml.	50 ml.
0.01-0.60 tungsten present	0.1	20 ml. of 70% perchloric acid 10 ml. of water 5-10 ml. of 85% phosphoric acid	50 ml. of water 10 ml. of 5% sodium thiocyanate	35 ml.	50 ml.
0.50-1.40	0.05	20 ml. of 70% perchloric acid 10 ml. of water	50 ml. of water 10 ml. of 5% sodium thiocyanate	15 ml.	50 ml.
1.4-7.0 tungsten present	0.1	20 ml. of 70% perchloric acid 10 ml. of water 10 ml. of 85% phosphoric acid	Dilute volumetrically to 100 ml., uso 10 ml. of sample as aliquot. Add 50 ml. of 30% perchloric acid and 10 ml. of 5% sodium thiocyanate	10 ml.	50 ml.

oxide fumes. Add 10 ml. of 10 per cent sodium hydroxide solution, evaporate to dryness, and ignite. When cool, take up with water and transfer to a 500-ml. flask. Dilute to volume. After complete sedimentation, use an aliquot of the supernatant layer for determination by thiocyanate.

Manganese-iron Ore. Dissolve 1 gram of finely powdered ore in 40 ml. of 1:1 hydrochloric acid. Boil gently until evolution of chlorine ceases. Filter and wash the filter with hot water. Evaporate the filtrate to dryness to eliminate excess hydrochloric acid. Take up the residue with 30 ml. of 1:100 hydrochloric acid. Precipitate manganese and iron by adding the solution to 20 ml. of hot 16 per cent sodium hydroxide solution in a 100 ml. flask. Filter through a dry filter and use an aliquot of the filtrate for the determination.

Tungsten-molybdenum Ores.[8] Transfer 0.5 gram of sample with 2.5 grams of sodium carbonate and 0.05 gram of sodium nitrate to a platinum crucible. Heat slowly, increasing the heat until a melt has formed. Extract the cooled melt with water containing a drop of ethanol to reduce manganate. Add some paper pulp, filter, and wash with warm 1 per cent sodium carbonate solution. Make the filtrate just acid with 1:4 hydrochloric acid. If chromium is present, add a few drops of saturated sulfurous acid solution to reduce the metal. Evaporate to about 25 ml. on the steam bath to remove carbon dioxide. Do not remove any silica that may separate out. This sample is designed for direct development with thiocyanate without extraction. At this point it may not be iron-free. Dilute to 100 ml. in a volumetric flask for the use of aliquots.

Alternatively,[9] fuse 0.5 gram of sample in an iron crucible with 2-3 grams of sodium hydroxide. Leach with hot water and add 2-3 ml. of 3 per cent hydrogen peroxide to the filtrate as oxidizing agent. Boil to decompose excess peroxide and cool. Transfer to a 250-ml. volumetric flask, dilute to volume, and use aliquots.

Ammonium Molybdate. Dissolve a 1-gram sample in 10 ml. of 10 per cent sodium hydroxide solution. Add about 40 ml. of water and boil until the volume is reduced to about 25 ml. Dilute to 1 liter and take an aliquot

[8] F. S. Grimaldi and R. C. Wells, *Ind. Eng. Chem., Anal. Ed.* **15**, 315-16 (1943).
[9] O. T. Bolotkova, *Zavodskaya Lab.* **8**, 324 (1939).

Molybdic Acid or Alkali Molybdates. Dissolve a 1-gram sample in 10 ml. of 10 per cent sodium hydroxide solution. Dilute to 1 liter and take an aliquot.

Tungsten and Molybdenum Deposits.[10] Small deposits on the walls of electric bulbs are not conveniently handled by fusion methods. Dissolve such deposits in 3 per cent hydrogen peroxide rendered slightly alkaline with ammonium hydroxide or slightly acid with nitric acid. Deposits of the metals or their oxides are readily soluble in either. Transfer the solution to a beaker and evaporate nearly to dryness to decompose the peroxide. During evaporation add about 10 drops of 1:1 hydrochloric acid to prevent precipitation. Take up with distilled water and dilute to 50 ml.

Minerals. A solution was prepared for determination of chromium (page 270). Use another aliquot for molybdenum.

Silicate Rocks.[11] Weigh 1 gram of 100-mesh rock powder into a platinum crucible and mix with 4-5 grams of anhydrous sodium carbonate. Cover and fuse. Continue heating for 30 minutes, or longer if much chromite or magnetite is present. Cool, cover with a few ml. of water, and warm gently to loosen the melt. Transfer to a small beaker, rinse the crucible with hot water, and add 2-5 drops of ethanol to reduce the manganate present. Add 30-40 ml. of water and heat on a steam bath with stirring to disintegrate the residue. Wash a filter paper with hot 20 per cent sodium carbonate solution and filter the sample solution into a 200-ml. volumetric flask. Wash any insoluble material in the beaker and filter with five 5-ml. portions of 1 per cent sodium carbonate solution. Neutralize and add 8 ml. excess of 1:3 hydrochloric acid. Separation of silica at this point may cause occlusion of molybdenum and cause low results. Dilute the filtrate and washings to volume, and aliquot for determination by potassium thiocyanate. In silicate rocks which may contain 0.001-0.0001 per cent of molybdic oxide, as little as 0.0001 per cent of molybdenum may be determined in a 1-gram sample by extraction of the thiocyanate with ether and by application of a duplication technic.

This method[12] tends toward retention of molybdenum in the residue. Furthermore, if such fusion is conducted in platinum, there is the

[10] W. Singleton, *Ind. Chemist* 2, 454-7 (1926).

[11] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 8, 336-41 (1936).

[12] M. L. Nichols and Lewis H. Rogers, *ibid.* 16, 137-40 (1944).

possibility that minute quantities of platinum may go into solution, causing interference.

Plants and Soils.[13] Dry the sample at 110°, then ash at 450° in a muffle furnace. Weigh 0.1-1.0 gram of the mixed, pulverized ash, and heat with 10 ml. of 1:4 hydrochloric acid. Filter, wash several times with water, and reserve the filtrate. Transfer the filter paper and residue to a platinum dish and ignite at 450°. Cool, add 5 ml. of water, 5 drops of concentrated sulfuric acid, and 10 ml. of 48 per cent hydrofluoric acid. Evaporate on a steam bath and ignite. Repeat the addition of water and hydrofluoric acid, re-evaporate, and ignite at 450°. This volatilizes the silica so that possible occlusion of molybdenum in the residue will be minimized. Cool, add 10 ml. of water and 1 ml. of concentrated hydrochloric acid, and warm. Filter if necessary, combine with the reserved filtrate and dilute to volume in a 50-ml. volumetric flask.

A method of preparation of plant tissue given under lead (page 30) yielded solution A suitable for molybdenum as well as iron, manganese, and phosphorus.

Biological Samples.[14] Transfer 2 grams of ground organs or 10 ml. of whole blood to a Pyrex test tube. Add 1 ml. each of concentrated sulfuric acid, concentrated nitric acid, and 70 per cent perchloric acid. Digest over a small flame and add more concentrated nitric acid as evidence of charring appears until the digestion is complete. Heat to perchloric acid fumes. Dilute to 10 ml. and add 1:1 ammonium hydroxide until alkaline to phenolphthalein. Cool to room temperature and add 4 ml. of concentrated hydrochloric acid. Dilute to about 15 ml. and add 1 ml. of a 0.04 per cent ferric chloride solution. This will serve to enhance the molybdenum color when developed with thiocyanate. Use the entire sample for determination by the extraction technic.

Standard

Dissolve 0.2522 gram of pure sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$, in 1 liter of 1·200 sulfuric acid. This contains 0.1 mg. of molybdenum per ml. Only in the most accurate work need it be standardized by reduction in a Jones reductor and titration with potassium permanganate.

[13] M. L. Nichols and Lewis H. Rogers, *ibid.* 16, 137-40 (1944); cf. F. B. Marmoy, *J. Soc. Chem. Ind.* 58, 275-6 (1939).

[14] D. D. Perrin, *New Zealand J. Sci. Tech.* 27A, 396-405 (1946).

Prepare further dilutions only as needed, since 0.001 per cent solutions of molybdenum are reduced to 20-40 per cent of their original strength after long storage in glass, owing to sorption and base-exchange reactions.[15] Ammonium molybdate may be used in place of the sodium salt. Dissolve 0.2042 gram of ammonium molybdate in water, and dilute to 1 liter. Each ml. contains 0.1 mg. of molybdenum.

Molybdenum trioxide may also be used to prepare standard solutions. Dissolve 1.5 grams of pure molybdenum trioxide in 10 ml. of 10 per cent sodium hydroxide solution and dilute to 1 liter. Each ml. contains 1 mg. of molybdenum. Dilute 100 ml. of the above to 1 liter to obtain a standard containing 0.1 mg. per ml. To prepare pure molybdenum trioxide for use as a standard, pass hydrogen sulfide into a faintly ammoniacal solution of commercial ammonium molybdate. Acidify with 1:1 sulfuric acid and filter the precipitate on a fine-grained filter. Wash with hot water until free from sulfates. Dry, separate from the filter, and roast in a porcelain crucible until white molybdenum trioxide is obtained. Keep in a desiccator. For standardization, 1.5 grams of the trioxide are equivalent to 1 gram of molybdenum.

Molybdenum by Thiocyanate

The amber to cherry-red color of molybdenum thiocyanate, obtained by adding an alkali thiocyanate to quinquevalent molybdenum in acid solution in the presence of a strong reducing agent such as stannous chloride,[16] is most often used in the determination of the metal. The color may be read directly in the solution in which it is developed, or may be extracted into organic solvent. The latter technic predominates.

The existence of two forms of the complex is indicated by a difference in slope of the line representing the absorption coefficient versus concentration at high and low levels.[17]

In aqueous solution, acetone [18] has a stabilizing action to prevent fluctuation of color with time. Glycol ethers such as butyl Cellosolve or butyl Carbitol in amounts of the order of 15 per cent are also advantageous in stabilizing the color.[19] Ammonium citrate helps to eliminate tungsten interference. If acid solutions are used, the acid concentration must be controlled. In the preferential extraction of quinquevalent molybdenum in a medium such as hydrochloric acid, pronounced color

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[15] Friedrich Leutwein, *Zentr. Mineral., Geol.* 1940A, 129-33.
[16] C. D. Braun, *Z. anal. Chem.* 2, 36 (1863).
[17] A. S. Shakhov, *Zavodskaya Lab.* 11, 391-4 (1945).
[18] F. S. Grimaldi and R. C. Wells, *Ind. Eng. Chem., Anal. Ed.* 15, 315-16 (1943).
[19] Mitchell Kapron and Paul L. Hehman, *ibid.* 17, 573-6 (1945).

changes occur as the concentration of the acid medium is altered.[20] In 1:5 hydrochloric acid which contains one equivalent of a reductant,[21] the color is amber, but in 2:1 acid solution the color turns to green. This reaction is reversible. Neutral salts and organic salts also are effective in shifting the color, hence the necessity of careful standardization of conditions. Heat serves to intensify the color of the solution. Both the acid solution and organic solvent extracts are read photometrically. The amber line has an absorption maximum at 460 mμ but readings are satisfactory at 420, 436, 475, 500 and 530 mμ.[22] It is desirable to make the reading within 10 minutes after addition of the reagents.[23]

A concentration of 10-15 per cent of sulfuric acid is optimum for the development and stabilization of the thiocyanate color within a reasonable period of time,[24] whereas 5 per cent is preferable in hydrochloric acid solution.[25] Similarly, if the color is to be read in perchloric acid solution, a minimum concentration of 17 per cent of the acid is required. Nitric acid is undesirable, since small amounts tend to increase the depth of the color. Potassium thiocyanate should be present at a concentration of not less than 0.6 per cent, and stannous chloride not less than 0.1 per cent. Stannic chloride resulting from reaction of stannous chloride with large amounts of ferric ion has some tendency to fade the color. When the acidity is substantially above or below the optimum, the color developed is less intense and fades more rapidly.

The color in aqueous acid solution which is not at optimum acidity fades rather quickly and therefore indicates the desirability of extraction, which also provides for concentrating. Water-immiscible organic solvents used include ethyl ether,[26] cyclohexanol,[27] and butyl acetate.[28] In ether, the color is approximately the same as that of the same concentration in 1:5 sulfuric acid.[29] In the use of ether, a drop or two of ferrous chloride may be used to improve the ether extraction. Small

[20] C. F. Hiskey and V. W. Meloche, *J. Am. Chem. Soc.* 62, 1565-74, 1819-24 (1940).

[21] F. Foerster, E. Fricke and R. Hausswald, *Z. physik. Chem.* Abt. A, 146, 81-100, 177-231 (1930).

[22] J. Pfanhauser and J. Jacewiczowna, *Przemt. Chem.* 20, 127-32 (1936).

[23] K. M. Popov and M. N. Dorokhova, *Zavodskaya Lab.* 9, 1315-17 (1940).

[24] H. Cox and A. A. Pollitt, *J. Soc. Chem. Ind.* 63, 375-8 (1944).

[25] Loren C. Hurd and Harry O. Allen, *Ind. Eng. Chem., Anal. Ed.* 7, 396-8 (1935).

[26] T. R. Cunningham and H. L. Hamner, *Ind. Eng. Chem., Anal. Ed.* 3, 106-7 (1931); Rudolf Sperl, *Chem.-Ztg.* 64, 363 (1940).

[27] Loren C. Hurd and Fred Reynolds, *Ind. Eng. Chem., Anal. Ed.* 6, 477-8 (1934).

[28] L. H. James, *ibid* 4, 89 90 (1932); David H. Heppell, *Ind. Chemist* 16, 173 (1940)

[29] John H Vail, *Chemist Analyst* 33, 52 62 (1944).

amounts of ether often exert an appreciable influence on the development of the thiocyanate color. Moreover, in ether solution, the presence of tungsten or vanadium causes high molybdenum results, unless tartrate or citrate ions are introduced.[30] Cyclohexanol exhibits more inert properties in that it does not exert any influence on the reaction and since it is a better solvent than ether, requires a smaller volume. It possesses a lower volatility and does not decompose at room temperature. Unlike butyl acetate, its hydrolysis products do not promote fading of the colored complex, nor does the color developed by a given concentration of molybdenum depend on the degree to which the solvent has been saturated with reagents. However, cyclohexanol possesses the disadvantage of separating rather slowly from aqueous solution and occasionally developing a turbid solution.

The molybdenum is generally reduced to the quinquevalent state with stannous chloride, although several other reducing agents have been tried, among them potassium chlorostannite, K_2SnCl_4,[31] sodium thiosulfate,[32] and titanous chloride.[33] There is always the possibility that the solvent used for extraction may contain peroxides. To test, shake with a fresh solution of potassium iodide and starch. If a blue color develops, shake the solvent with 10 per cent sodium thiosulfate and distill. The iron present is reduced to the ferrous state with stannous chloride, thus avoiding the characteristic red of ferric thiocyanate.

If rhenium is present, it too is reduced in the determination of molybdenum and interferes. However, if a dilute hydrochloric acid solution is reduced with mercury, the molybdenum is reduced to the valence required for the formation of the colored compound, but rhenium is not affected. After extracting the molybdenum with ether and treating with potassium thiocyanate, the colorimetric determination must be made immediately, because the color is quite fugitive. Rhenium may be determined in the residual solution[34] in the usual way with thiocyanate and stannous chloride. In 3:200 hydrochloric acid, large amounts of molybdenum tend to form a blue compound which does not react with thiocyanate; therefore this concentration is used to determine small amounts of molybdenum in the presence of relatively large amounts of rhenium. In 1:4 hydrochloric acid, the blue complex is not formed,

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[30] F. S. Grimaldi and R. C. Wells, *Ind. Eng. Chem., Anal. Ed.* **15**, 315-16 (1943).

[31] Kurt Dietrich and Karl Schmitt, *Metallwirtschaft* **17**, 88-9 (1938).

[32] Kenneth E. Stanfield, *Ind. Eng. Chem., Anal. Ed.* **7**, 273-4 (1935).

[33] F. A. Fer'yanchich, *Zavodskaya Lab.* **6**, 289-92 (1937).

[34] James I. Hoffman and G. E. F. Lundell, *J. Research Natl. Bur. Standards* **23**, 497-503 (1939).

but a trace of rhenium may be reduced and react with the thiocyanate; however, large amounts of molybdenum in the presence of small quantities of rhenium may be determined in this solution. Platinum, rhodium, vanadium, tungsten and large amounts of chromium interfere. Large amounts of copper may precipitate as the thiocyanate and necessitate filtration.

When reading the color of an aqueous solution, addition of 1 per cent of chromium showed up as 0.008 per cent of molybdenum. Thus the error is not serious up to 2 per cent of chromium.[35] With a separately determined value for chromium it is satisfactory to correct by subtraction. Large amounts of vanadium may become troublesome because of the blue-green color of the reduced vanadium ion.[36]

If tungsten is absent, the presence of phosphate ions does not present any difficulty; if it is present, an excess of ammonium citrate may be used to minimize the interference. A high fluoride content may lead to low results. The presence of arsenic, antimony, carbon, nickel, cobalt, manganese, titanium, zirconium, aluminum, silicon, and less than 0.13 per cent of copper, does not interfere. Interfering amounts of chromium can be distilled out as the chloride but extraction of the developed color is simpler.

The presence of iron increases the intensity due to molybdenum. The effect is not so much a function of the quantity of iron present as of the time elapsed before the sample is matched.[37] This effect reaches a maximum in the presence of 2 mg. of iron per 100 ml. and additional iron does not have a further effect.[38] Iron[39] and chromium[40] may be separated by precipitation with sodium hydroxide. However, since the iron thiocyanate complex is quickly destroyed by the stannous chloride, removal is not necessary.[41]

The determination of 0.1-0.6 per cent of molybdenum may be made with an accuracy of 0.01 per cent, whereas the estimation of 0.6-2 per cent is accurate to 0.03 per cent.

Procedure. *Direct Reading with Iron Removal.*[42] Although a

[35] John H. Vail, *Chemist Analyst* **33**, 52-62 (1944).
[36] A. Accardo and F. Abramo, *Congr. chim. ind., Compt. rend. 18me congr., Nancy*, Sept. Oct., 1938, 138-41.
[37] F. S. Grimaldi and R. C. Wells, *Ind. Eng. Chem., Anal. Ed.* **15**, 315-16 (1943).
[38] M. L. Nichols and Lewis H. Rogers, *ibid.* **16**, 137-40 (1944).
[39] O. Keune, *Tech. Mitt. Krupp* **3**, 215-18 (1935).
[40] I. Sokolova, *Atsapromyshlennost* 1939, No. 3, 52-5.
[41] A. Eder, *Arch. Eisenhüttenw.* **11**, 185-7 (1937).
[42] F. S. Grimaldi and R. C. Wells, *Ind. Eng. Chem., Anal. Ed.* **15**, 315-6 (1943).

complex-forming salt is later added, the presence of iron will interfere in this technic. Therefore it must first be removed. Take an aliquot of sample to contain 0.2-2.0 mg. of molybdenum. Neutralize with 10 per cent sodium hydroxide solution and add 0.5 ml. in excess. Add a little paper pulp and digest on a steam bath for 10 minutes. Filter while hot and wash with 1 per cent sodium hydroxide solution. Add a drop of phenolphthalein solution to the filtrate and washings and make barely acid with 1:4 hydrochloric acid. Adjust the volume to 15 ml. Add 1.5 grams of ammonium citrate and stir until dissolved. Add 5 ml. of 10 per cent ammonium thiocyanate solution and 25 ml. of acetone. Cool and add dropwise exactly 7 ml. of 10 per cent stannous chloride dihydrate in concentrated hydrochloric acid. The amber color of molybdenum thiocyanate develops at once. Filter if silica is precipitated at this step.

Prepare a blank containing 15 ml. of water, 1.5 grams of ammonium citrate, 5 ml. of 10 per cent ammonium thiocyanate, and 25 ml. of acetone. Add exactly 7 ml. of the 10 per cent stannous chloride solution and, when well mixed, add a standard containing 0.1 mg. of molybdenum per ml. until the color is matched.

Without Iron Removal.[43] Dilute or concentrate a sample containing 0.2-2.0 mg. of molybdenum to about 10 ml. and transfer it to a 100-ml. volumetric flask. Add 70 ml. of 1:1 sulfuric acid and 3 ml. of 50 per cent potassium thiocyanate solution. Add 5 ml. of a 10 per cent solution of stannous chloride in 1:1 hydrochloric acid. Dilute to volume and read 10 minutes later. This may be by comparison with a parellel standard or by reading photometrically at 450-480 $m\mu$.

Extraction. As aliquot, take a portion of sample containing 0.1-0.5 mg. of molybdenum. Samples prepared from steels will ordinarily have the proper acidity. For others if the sulfuric acid content is approximately 10 per cent, use as is. When the acidity is due to perchloric acid, 17 per cent is a minimum concentration of acid. Adjust the acidity if it falls below the required minimum for the acid in question. If in doubt titrate the aliquot to approximate neutrality with 8 per cent sodium hydroxide solution or 1:3 sulfuric acid. Add approximately one-fifth the volume of 1:1 sulfuric acid. If necessary to dilute, use 1:10 sulfuric acid. Transfer[44] this solution to a 25-ml. separatory funnel and, unless it is to be read photoelectrically, a standard of approximate

[43] A. M. Gulva, *Zavodskaya Lab.* **11**, 473 (1945); cf. N. A. Tananaev and A. P. Lokhvitskaya, *ibid.* **11**, 6-10 (1945).

[44] Arba Thomas, *Proc. Am. Soc. Testing Materials* **44**, 769-78 (1944).

equivalence to another such funnel. Table 6 (page 474) shows the amounts of all reagents for some alloy steels.

If the sample is a steel, use as standard a similar steel of known molybdenum content, dissolved in the same way as the sample, or add standard molybdenum solution to a molybdenum-free steel. If sample and standard do not already contain iron, add 1 ml. of a 2.5 per cent solution of ferric sulfate to each. To each add 10 ml. of a 5 per cent solution of sodium thiocyanate and shake for 30 seconds. Add 5 ml. of a solution of 35 grams of hydrated stannous chloride in 20 ml. of 1:1 hydrochloric acid, diluted to 100 ml., and kept reduced with metallic tin. The use of too much stannous chloride appears to cause rapid fading of the color. Therefore a rough proportionality must be followed in increase or decrease of size of steel samples. Shake well for 1 minute, cool to room temperature, and add 20 ml. of butyl acetate previously saturated with sodium thiocyanate and stannous chloride by shaking. Shake well, draw off the lower layers which separate, and discard those layers. Compare the colors of the two extracts. This may be by dilution or balancing without restriction. The color may not fully conform to Beer's law; therefore for the balancing method the difference between sample and standard should not exceed 25 per cent. Ethyl ether is an alternative extraction medium but offers greater difficulty in manipulation as would be expected from its greater volatility.

The color of the solvent extract of the molybdenum thiocyanate complex may be matched by a permanent artificial standard prepared from a nearly saturated potassium dichromate solution,[45] or from methyl orange.[46] Instead of comparing with a natural or artificial standard, it is satisfactory to read the absorption photoelectrically with a filter centering around 520 mμ and compare with a standard curve.[47]

Separation of Molybdenum in the Presence of Rhenium.[48] Because of the close relation of rhenium and molybdenum, a method of separation is necessary.

Adjust the acidity and volume of the sample solution so that 10-20 ml. in 1:1 hydrochloric acid can be used. Add a saturated solution of potassium permanganate dropwise to the cold solution until in excess, so that molybdenum will be present as molybdate, rhenium as perrhe-

[45] John H. Vail, *Chemist-Analyst* **31**, 80 (1942).

[46] F. A. Fer'yanchich and D. N. Jordanskii, *Zavodskaya Lab.* **7**, 866-7 (1938).

[47] G. M. Poole, *Iron Age* **148**, No. 15, 62, 164 5 (1941).

[48] James I. Hoffman and G. E. F. Lundell, *J. Research Natl. Bur. Standards* **23**, 497 508 (1939).

nate. Add 1 ml. of a 2 per cent solution of ferric chloride unless at least 10 mg. of iron is already present. Transfer to a separatory funnel and add 20 ml. of ethyl ether. Shake for about 1 minute, let separate, and withdraw the acid layer into another separatory funnel. Repeat the extraction twice with 10-ml. portions of ether. Reserve the acid layer. Add 10 ml. of 1:1 hydrochloric acid to the combined ether extracts and shake for a few seconds. Let separate and add the acid washings to the previous acid layer. The bulk of the molyhdenum is now in the ether layer, of the rhenium in the aqueous acid layer. Thus with 2 mg. each of molyhdenum and rhenium in the original sample, there was 0.12 mg. of rhenium in the ether layer and 0.09 mg. of molybdenum in the acid extract. In, separation of 10 mg. of each there was 0.29 mg. of rhenium in the etber layer and 0.8 mg. of molybdenum with the acid extract.

Evaporate tbe aoid layer nearly to dryness on a steam bath. Add 2-3 drops of saturated potassium permanganate solution to tbe nearly dry residue. Add sufficient 1:1 ammonium hydroxide to make the residue definitely alkaline and heat for a few minutes to insure oxidation. Take up the residue in 25 ml. of 3:200 hydrochloric acid. Add 2 ml. of a 20 per cent solution of potassium thiocyanate and transfer to a separatory funnel containing ahout 25 grams of mercury. Add 20 ml. of ether, stopper, and shake until the solution over the mercury has lost the color of ferric thiocyanate. This usually requires not more than 1 minute. Witbdraw the mercnry and acid to another separatory funnel, reserving the ether solution of molybdenum. Add 1 ml. of 20 per cent potassium thiocyanate solution and 15 ml. of ether to the acid layer and mercury. Shake for 1 minute and separate. Repeat the extraction a third time. Set aside tbese comhined ether extracts as containing molybdenum substantially free from rhenium and this acid layer as rhenium suhstantially free from molyhdenum.

Evaporate tbe ether extract containing molyhdenum with some rhenium as ohtained from the first separation. Add 1 ml. of 2 per cent ferric chloride solution and proceed as with the acid layer from the rough separation, starting at "Add 2-3 drops of saturated potassium permangate solution . . ." The end product will he an ether extract of molybdenum, suhstantially free from rhenium, and an acid solution of rhenium substantially free from molyhdenum.

Comhine the ether solutions of molybdenum and wash with 10 ml. of 1:4 hydrochloric acid. Prepare ether for dilution by shaking 100-200 ml. with 25 ml. of 1:4 hydrochloric acid, 2 ml. of 20 per cent potassium thiocyanate solution and 10 grams of mercury. Dilute the ether solution

of molybdenum to a known volume and read the color. Reserve the rhenium solution for determination by the same principle (page 542).

MOLYBDENUM BY POTASSIUM ETHYL XANTHATE

Molybdenum may be determined by adding potassium ethyl xanthate, $KS_2COC_2H_5$, to an acid solution and extracting the red-violet color with a mixture of petroleum and ethyl ethers, or with chloroform.[49] The pH of the solution preferably should be 1.8-1.9.[50] The intensity of the color is proportional to the concentration. The method is accurate to 0.8 per cent and is useful for determining 0.01 mg. or less of molybdenum. Chromium, nickel, manganese, and vanadium do not interfere.[51]

The use of the xanthate method is not consistently satisfactory,[52] probably because part of the molybdenum is converted to a blue compound which cannot be extracted with chloroform.[53] The method is used for determination of molybdenum in pure solutions,[54] but not in steel.[55] Phosphates interfere and there is no advantage over the thiocyanate method.

Procedure. As reagent, shake carbon disulfide in excess with 100 ml. of a saturated solution of potassium hydroxide in absolute ethanol until no further reaction occurs. Leave a small amount of carbon disulfide in the bottom to insure saturation. To an aliquot of sample, add 1 per cent sodium hydroxide solution to faint alkalinity. Add 5 ml. of reagent, mix well, and add 30 per cent acetic acid until distinctly acid. Extract with 25 ml. of a mixture of 35 per cent petroleum ether and 65 per cent ethyl ether, or with 25 ml. of chloroform. Repeat with another 25 ml. portion of solvent. Mix the two colored extracts and compare, by dilution with solvent, with a standard prepared by similar treatment.

MOLYBDENUM BY HYDROGEN PEROXIDE

Salts of molybdic acid, in alkaline solution, may be oxidized with

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49 H. Leitmeier and F. Feigl, *Mineralog. petrog. Mitt.* 47, 313-27 (1936).

50 F. Pavelka and A. Laghi, *Mikrochemie verein. Mikrochim. Acta* 31, 138-44 (1943).

51 G. A. Panchenko, *J. Applied Chem.* (U.S.S.R.) 8, 722-6 (1935).

52 E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 8, 336-41 (1936); P. Klinger, *Arch. Eisenhüttenw.* 14, 157-77 (1940).

53 James I. Hoffman and G. E. F. Lundell, *J. Research Natl. Bur. Standards* 23, 497-508 (1939).

54 P. L. Hoogland and G. A. Lampe, *Arch. Néerland. physiol.* 27, 145-64 (1943).

55 P. Klinger, *Arch. Eisenhüttenw.* 14, 157-77 (1940).

hydrogen peroxide to permolybdates, reddish brown in color.[56] As the intensity of the color is also proportional to the concentration of hydrogen peroxide, particular care must be taken to keep the latter constant. A variation in the amount of free alkali does not affect the color. In acid solution, hydrogen peroxide forms a pale yellow color with molybdenum ions, which is intensified by phosphoric acid.[57] An intense absorption peak occurs at 330 mμ.[58]

The method is inapplicable in the presence of salts of chromium and tungsten. Ammonium salts must be decomposed by boiling with sodium hydroxide. In any case permolybdates are unstable so that the color is destroyed slowly on standing. A distinct reaction is obtained with 0.025 mg. of molybdic oxide.

Procedure. *Titanium and Vanadium Absent.* Select an aliquot containing about 1-3 mg. of molybdenum. Neutralize with 10 per cent sodium hydroxide and add 5 ml. in excess. Dilute or concentrate to about 40 ml. Add 2 ml. of 3 per cent hydrogen peroxide and, if the color is not sufficiently intense, increase the reagent to 10 ml. Alternatively, use 2 ml. of 30 per cent hydrogen peroxide. Dilute to 50 ml. and compare with standards similarly prepared, or read photometrically.

Molybdenum, Titanium, and Vanadium Together. The method is given in detail under titanium (page 442).

Miscellaneous

Hexavalent molybdenum may be determined colorimetrically by treating a solution of sample with pyrogallol, 1,2,3-trihydroxybenzene.[59] The resulting yellow to orange-red color produced by oxidation increases in intensity with the molybdenum concentration and varies with pH. At the optimum pH of 4.4, the color is stable for 1 hour. Iron and tungsten must be absent. To an aliquot of solution to be tested, add 2 drops of 0.1 per cent *p*-nitrophenol solution. If the solution turns yellow, decolorize with 1:10 hydrochloric acid; if it is originally colorless, titrate with 4 per cent sodium hydroxide solution to a yellow color and decolorize with a drop of 1:10 hydrochloric acid. Dilute the solution so neu-

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[56] A. D. Funck, *Z. anal. Chem.* 68, 283-6 (1926).

[57] G. Thanheiser and P. Göbbels, *Mitt. Kaiser-Wilhelm-Inst. Eisenforsch Dusseldorf* 23, 187-94 (1941).

[58] Alfred Weissler, *Ind. Eng. Chem., Anal. Ed.* 17, 695-8 (1945).

[59] R. I. Alekseev, *Zavodskaya Lab.* 7, 863-5 (1938).

tralized to a volume which will contain 0.05-0.1 mg. of molybdenum per ml. To a 10 ml. aliquot, add 10 ml. of pyrogallol solution containing 1 per cent of pyrogallol and 2 per cent of glacial acetic acid. Add 10 ml. of a 3 per cent solution of sodium acetate trihydrate, mix, and compare with similarly prepared standards by balancing or duplication methods.

By a similar reaction, the addition of dilute tannic acid to an acetic acid solution of molybdenum gives a color suitable for estimation of the molybdenum content.[60] Neutralize a suitable volume of sample, then add 10 ml. of glacial acetic acid and dilute to about 45 ml. Transfer 2 ml. of fresh 0.5 per cent tannic-acid solution to a 50-ml. Nessler tube. Add the solution of sample, mix well, and dilute to volume. Compare against similarly prepared standards or use the duplication method.

A sensitive test for molybdenum involves its reduction in the presence of α,α'-bipyridyl with chlorostannous acid.[61] Even more sensitive results are obtained in the presence of 1,10-phenanthroline, 5-chloro-1,10-phenanthroline, 5-bromo-1,10-phenanthroline, 5-methyl-1,10-phenanthroline, nitro-1 10-phenanthroline, or 5-nitro-6-methyl-1,10-phenanthroline.[62] However, the reddish purple color obtained is quite unstable, fades readily, and displays poor reproducibility.

A saturated aqueous solution of phenylhydrazine, to which mineral acid has been added, gives a pink to blood-red color on warming with molybdenum solutions.[63] Iodates give free iodine, persulfates a faint yellow, vanadates green; permanganates are decolorized. Perchlorates, perborates, and tungstates are without effect. The solution must be acid to avoid precipitation. Apparently the reaction is specific for molybdenum. The color is stable for several hours and will detect 0.02 mg. of molybdenum. However, the method is not sufficiently sensitive to detect very minute amounts of molybdenum.

To 10 ml. of the sample in sulfuric acid solution, add 5 ml. of a reagent containing 5 per cent each of phenylhydrazine and concentrated sulfuric acid. Heat on a water bath to 80° for 15 minutes, cool for 30 minutes, and compare with a similarly developed standard. Hydrazine sulfate may replace the phenylhydrazine.[64]

Colloidal molybdenum sulfide varies according to concentration, from

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[60] G. Spurge, *Chem. Eng. Mining Rev.* 11, 258 (1919).

[61] A. S. Komarovskii and N. S. Poluektov, *Mikrochim. Acta* 1, 264-6 (1937).

[62] M. L. Moss, M. G. Mellon and G. Frederick Smith, *Ind. Eng. Chem., Anal. Ed.* 14, 931-3 (1942).

[63] E. Montignie, *Bull. soc. chim.* [4] 47, 128 (1930); cf. P. Klinger, *Arch. Eisenhüttenw.* 14, 157-77 (1940).

[64] N. M. Miloslavskii and E. G. Vavilova, *Zavodskaya Lab.* 5, 12-16 (1936).

yellow to brownish red. Molybdenum in molybdates or oxides may be estimated by means of the color of the sulfide dispersion.[65] The method is exceedingly delicate, the difference in color between 0.01 and 0.02 mg. being easily perceptible by using a modified micro form of the method. Mix 5 ml. of glycerine with 100 ml. of water and saturate with hydrogen sulfide. The glycerine prevents subsequent precipitation of sulfur. To 25 ml. of sample in a tube and 25 ml. of standard in another, add 15 ml. of water and 5 ml. of the hydrogen sulfide solution. Add 1 per cent sulfuric acid dropwise to each solution with constant stirring until the color of the more concentrated is no longer deepened. Dilute each to 50 ml. and compare by balancing.

When excess of sodium thiosulfate solution is added to a molybdate solution acidified with hydrochloric acid, a precipitate of sulfur separates and the molybdenum is reduced to give a color extractable with ether or ethyl acetate. The color in the organic solvent is suited to colorimetric estimation against a series of standards.[66] The color varies with concentration from lilac to rose to red-brown. Five mg. of molybdic acid give a red color, 0.005 mg. a lilac. The reaction is more sensitive than those with stannous chloride, hydrogen peroxide or potassium xanthate. Alkali destroys the color. The color is not affected by tartaric, citric, oxalic or tannic acids, ammonium salts, chromates, chlorates, and many cations. Copper and iron in sufficient concentration may interfere. Add 2 ml. of concentrated hydrochloric acid to 10 ml. of the sample solution and extract with 8 ml. of a mixture of 80 per cent ethyl acetate and 20 per cent ether. Shake the solvent layer with an equal volume of 30 per cent sodium thiosulfate solution. Separate the layers and repeat with fresh thiosulfate solution. Filter the solvent layer and compare with a series developed from standards.

The reducing action of stannous chloride on molybdenum and tungsten in hydrochloric and phosphoric acids causes a light blue to deep violet color proportional to the molybdenum content.[67] The reaction is suitable for estimation of traces of molybdenum in steel. Vanadium must be absent. It is conveniently removed by reducing the vanadium and part of the iron with sodium bisulfite and precipitating with sodium hydroxide. Tungsten may be eliminated by oxidation with chlorate and filtration from insoluble tungstic acid. The method will detect 1 part in 8 million.

Prepare a tungstic acid reagent by dissolving 11.4 grams of ammo-

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[65] E. Wendehorst, *Z. anorg. Chem.* **144**, 319-20 (1925).

[66] Pietro Falciola, *Ann. chim. applicata* **17**, 261-2 (1927).

[67] Ernest Bertrand, *Chimie et Industrie* **198**, Special No. March, 1931.

nium tungstate containing 70 per cent of tungsten, in 40 ml. of 10 per cent sodium hydroxide solution. Heat if necessary. Add 70 ml. of 30 per cent tartaric acid solution and 5 ml. of concentrated hydrochloric acid. Dilute to 500 ml. and pass hydrogen sulfide through the solution for 30 minutes. After standing 12 hours filter out molybdenum sulfide originally present as molybdate in the tungsten and wash the precipitate. Boil off the hydrogen sulfide from the solution and when cool dilute to 2 liters. To a 50-ml. aliquot of sample, add 6 ml. of 1:1 hydrochloric acid, 1 ml. of 85 per cent orthophosphoric acid and 5 ml. of the tungstic acid reagent. Dilute to about 80 ml. and add 10 ml. of a stannous chloride solution, produced by dissolving 20 grams of tin in 200 ml. of concentrated hydrochloric acid, and diluting to 1 liter. Dilute to 100 ml. Compare the color which develops at once, with that produced by similar treatment of a suitable standard. The color is very stable.

The addition of a 1 per cent solution of hematoxylin to a sample made slightly alkaline with sodium hydroxide results in the formation of a complex that may be utilized for colorimetric determination.[68]

Molybdenum reacts to give a characteristic green color with toluene-3,4-dithiol which is extractable with amyl acetate.[69] To an oxidized sample containing 4 mg. of molybdenum in 3 ml. of 1:2 hydrochloric acid add 3 ml. of 1 per cent solution of toluene-3,4-dithiol in amyl acetate and let the mixture stand for 15 minutes of occasional shaking. Separate the insoluble solvent layer and wash with an equal volume of concentrated hydrochloric acid. Dilute with solvent to 10 ml. and read the transmittance at an appropriate wave length.

[68] V. Ya Tartakovskii, *Zavodskaya Lab.* 9, 971-5 (1940).
[69] J. E. Wells and R. Pemberton, *Analyst* 72, 185-8 (1947).

CHAPTER 27

URANIUM

THIS ELEMENT has suddenly leaped from obscurity to promiuence. The amounts in minerals are not relatively large but small occurrences may be important, particularly in relation to future power supplies by atomic fission. There is considerable unpublished work in the field. The methods given represent the prewar status. In fact as of that period, and it is a measure of importance of the element at that time, methods for determination of uranium were much more consequential for estimation of sodium indirectly as sodium uranyl acetate than directly for uranium. The methods given are rather heterogeneous because none has general acceptance.

SAMPLES

Low Grade Uranium Ore. Weigh 0.5 gram of ore and, if it contains much carbonate, wet the sample with 5 ml. of water. Add 20 ml. of 1:4 sulfuric acid with care to prevent loss from spattering. Add 5 ml. of 1:2 hydrochloric acid and boil gently for one-half hour. Dilute the solution to 50 ml. with hot water and filter. Wash the residue with hot 1:4 sulfuric acid.

Make the filtrate and washings alkaline with 1:1 carbonate-free ammonium hydroxide and add 4-5 ml. of 3 per cent hydrogen peroxide solution. This precipitates the metals as sesquioxides. Filter and wash the precipitate with a hot 3 per cent solution of ammonium sulfate containing a few drops of concentrated ammonium hydroxide. Dissolve the precipitate in the smallest possible volume of hot 1:100 sulfuric acid. The total volume should not be over 50 ml.

Transfer this solution, which now contains uranium, iron, aluminum, and possibly vanadium, to an electrolysis vessel for the separation of iron (page 2366). Electrolyze, using a mercury cathode, with a current of 4-5 amperes and 6-8 volts. Test for iron in the solution with a 0.2 per cent solution of potassium ferricyanide on a spot plate. When the solution is free from iron, transfer to a beaker, rinsing out the electrolysis chamber. The total volume of solution should not be over 100 ml. at this point.

Precipitate uranium and aluminum as before, with ammonium hydroxide in the presence of hydrogen peroxide. If vanadium is present, uranium is precipitated mostly as uranium vanadate. Wash the precipitate 3-4 times with a hot 3 per cent ammonium sulfate solution containing a few drops of concentrated ammonium hydroxide. If the ore contains no vanadium, dissolve the ammonium uranate precipitate in 0.2 per cent sulfuric acid. Test the solution for iron. If free from iron, make up to 100 ml. with the same acid.

If vanadium is present, dissolve the precipitate in 1:50 sulfuric acid. Neutralize with 1:2 ammonium hydroxide until a permanent turbidity remains. Remove the turbidity by addition of a few drops of 1:20 sulfuric acid. Be careful to avoid using any more acid than is necessary to make the solution clear. Dilute to about 40 ml.

To the sulfuric acid solution add 5 ml. of 1:2 acetic acid and 15 ml. of 4.5 per cent disodium phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$, solution. If the amount of aluminum present is very small, coagulation is improved by the addition of 2 ml. of a 5 per cent solution of potassium aluminum sulfate. Aluminum helps carry down uranium phosphate. Heat to boiling and let stand for 10 minutes. Filter and wash 5-6 times with 8 per cent ammonium nitrate solution. Vanadium is completely removed in the filtrate and washings. Dissolve the uranium phosphate precipitate in 25 ml. of a suitable type and concentration of acid according to the method of analysis to be used. Make up to 100 ml. with the same acid.

Organic Matter. The amonnt of sample taken should correspond to 2-6 mg. of uranium oxide, U_3O_8. Ash the sample hy any convenient method. Dissolve the ash in a mixture of hydrochloric and nitric acids. If much calcium is present, add snlfuric acid to precipitate it as the sulfate. Filter and wash the precipitate. Separate uranium from the filtrate as given in detail for ore (page 490) starting at "Make the filtrate and washings alkaline . . ."

Standard

Dissolve 1.7885 grams of iron-free uranyl nitrate in water and dilute to 1 liter. This corresponds to abont 1 mg. of uranium oxide, U_3O_8, per ml. Determine the concentration of uranium exactly hy gravimetric analysis. Precipitate uranium from 50 ml. of this solution with 1:1 carbonate-free ammonium hydroxide in the presence of 4-5 ml. of 3 per cent hydrogen peroxide solution. Wash the precipitate with 3 per cent

CHAPTER 28

COLUMBIUM

Columbium, also known as niobium, occurs in mineral deposits, very often in combination with tantalum. Small quantities of columbium incorporated in rustless steels form stable carbides that prevent grain disintegration in austenitic chromium-nickel steels.[1] The available methods are strictly limited; these are by pyrogallol, by hydrogen peroxide, and by reduction of the fluoride. The first possesses the advantage that tantalum does not interfere. The properties of the ion are in general like those of titanium.

Samples

Tantalum.[2] Transfer a 0.1-gram sample to a platinum dish and add 2 ml. of 1:4 nitric acid and a drop of 48 per cent hydrofluoric acid. Warm and, if necessary, add more hydrofluoric acid, finally obtaining a clear solution. Dilute to a known volume for use of an aliquot.

Oxides of Columbium, Tantalum, etc.[3] Fuse a sample containing about 0.01 gram of columbic oxide with excess sodium pyrosulfate. Let cool and take up in about 70 ml. of saturated ammonium oxalate solution acidified with 2-4 ml. of 1:1 sulfuric acid. Filter and wash the residue. Dilute the filtrate to 100 ml., and use an aliquot.

Tantalite. Dissolve 10 grams of sample in 80 ml. of 1:3 hydrochloric acid with the addition of 15 ml. of 48 per cent hydrofluoric acid. Evaporate the clear solution to 40 ml. Large amounts of potassium fluotantalate will separate. Cool, filter, and wash the residue with 10 ml. of cold water containing 0.5 ml. of 48 per cent hydrofluoric acid. Potassium fluocolumbate remains in solution with a small amount of fluotantalate. Evaporate carefully to dryness on the sand bath. Dissolve in 10 ml. of concentrated hydrochloric acid. Rinse into a volumetric flask with

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[1] P. Klinger, *Tech. Mitt. Krupp Forschungsber.* **2**, 171-3 (1939).

[2] P. Klinger, E. Stengel and H. Wirtz, *Metall u. Erz* **38**, 124-7 (1931).

[3] M. S. Platonov and N. F. Krivoshlykov, *Trudy Vsesoyuz. Konferentsii Anal. Khim.* **2**, 359-70 (1943).

5 ml. of water and dilute to 25 ml. with concentrated hydrochloric acid. If the sample contains more than 0.1 per cent of columbium, the amount of sample should be correspondingly reduced. This method of preparation is especially suitable if columbium is to be determined by reduction of the fluoride.

Minerals and Ores. If a high iron content is present, fuse a 2-gram sample with potassium hydroxide.[4] Take up the cooled melt in water and dilute sufficiently to filter through paper. Wash the residue on the filter thoroughly. Add 1:1 sulfuric acid to the filtrate until it is faintly acid and dilute to 100 ml. for the use of aliquots.

If iron does not interfere, add a 2-gram sample to 10 ml. of concentrated sulfuric acid and 2 ml. of 48 per cent hydrofluoric acid, and heat slowly until the sample has decomposed. Let cool and take up in water. Filter, wash the residue, and dilute to 100 ml. for the use of aliquots.

Alternatively, fuse 0.1 gram of sample with 1 gram of sodium pyrosulfate. Cool and leach the melt with a saturated ammonium oxalate solution containing 1-2 ml. of concentrated sulfuric acid. Dilute to an appropriate volume and use aliquots, determining preferably by pyrogallol.

Standard

Fuse 0.01 gram of columbium pentoxide with 1 gram of sodium pyrosulfate, $Na_2S_2O_7$, cool, and dissolve in 70-80 ml. of saturated ammonium oxalate solution. Dilute to 100 ml. Each ml. is equivalent to 0.1 mg. of columbium pentoxide.

Columbium by Pyrogallol

The addition of pyrogallol to alkaline solutions containing columbium in the presence of sodium sulfite results in a stable yellow color. Under the same conditions but in an acid medium, a similar color appears only if tantalum is present.[5] Increasing the pyrogallol concentration increases the sensitivity and intensity of the reaction, but decreases the stability of the color due to oxidation of pyrogallol.[6] The amount pres-

[4] N. V. Alekseevskaya and M. S. Platonov, *J. Applied Chem.* (U.S.S.R.) 10, 2139-42 (1937).

[5] N. P. Krivoshlykov and M. S. Platonov, *ibid.* (U.S.S.R.) 10, 184-91 (1937).

[6] M. S. Platonov and N. P. Krivoshlykov, *Trudy Vsesoyuz. Konferentsii Anal. Khim.* 2, 359-70 (1943).

ent in sample and standard must be rigidly standardized. Columbium present to 0.5 mg. may be detected in 1 ml. of solution.[7]

Procedure. Measure out an aliquot of solution containing columbium, with or without tantalum. Add 10 per cent sodium hydroxide solution until approximately neutral, then 10 ml. of saturated sodium sulfite solution. At this point the sample solution should be distinctly alkaline. Add 10 ml. of 8 per cent pyrogallol solution and dilute to 50 ml. Compare the color produced with similarly treated standards, or against permanent standards prepared from ammonium chromate solutions. The balancing method is suitable. The same solution may be used for determination of tantalum (page 500).

If titanium is present, the results are too high. Determine it separately by the hydrogen peroxide method and apply a correction. The color of a given weight of titanium dioxide is equal to that from 60 per cent of the weight of columbic oxide. Therefore multiply the titanium determined by 1.66 and subtract.

Columbium by Hydrogen Peroxide

Columbium gives the familiar yellow color with hydrogen peroxide which is customarily used for estimation of titanium, but only in solutions of sulfuric acid stronger than 20 per cent.[8] Titanium gives the same color unless the concentration of acid is carried into the fuming concentrations, when the color is lessened. It is more convenient to determine the two elements in 100 per cent sulfuric acid and subtract a correction for titanium. Tantalum gives no color. In a 2:3 phosphoric acid-sulfuric acid solution, columbium can be determined in the presence of up to 1 per cent of titanium without correction.[9]

Procedure. Transfer a suitable aliquot, which may contain titanium as well, to a platinum dish. Add 4 ml. of concentrated sulfuric acid and evaporate to strong sulfur trioxide fumes. Add 3 per cent hydrogen peroxide to the cooled solution, dilute to 20 ml., and read the transmittance with a 436-mμ filter in light from a mercury arc. This measures the titanium present.

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[7] N. F. Krivoshlykov, *Trudy LKKhTI* **1939**, No. 7, 103-22; *Khim. Referat. Zhur.* **2**, No. 5, 59-60 (1939).

[8] P. Klinger and W. Koch, *Tech. Mitt. Krupp Forschungsber.* **2**, No. 14, 179-85 (1939); *Arch. Eisenhüttenw.* **13**, 127-34 (1939).

[9] G. Thanheiser, *Mitt. Kaiser-Wilhelm-Inst. Eisenforsch. Düsseldorf* **22**, 255-65 (1940).

Re-evaporate the solution to fumes of sulfur trioxide and take up the cooled residue with slightly fuming sulfuric acid, d. 1.845/15°. Dilute to 20 ml. with the same acid and take a 10-ml. portion for color development. To this add 0.1 ml. of 30 per cent hydrogen peroxide, mix well, and read the yellow color with the 436-mμ filter as before. Translate the reading into columbium and subtract the titanium previously determined on the basis of 1 mg. of titanium being equivalent to 1.66 mg. of columbium. The 1 per cent error introduced by the reagent is insignificant.

Columbium by Reduction of the Fluoride

Reduction of the blue fluoride compound of columbium with zinc in the presence of hydrochloric acid results in a change to brown. This may be used as a basis for colorimetric determination without interference.[10] The method is applicable to as low as 0.005 per cent of columbium. The glassware used will be etched by the hydrofluoric acid. Test tubes calibrated by the operator are therefore recommended for comparison.

Procedure. Reduce an aliquot of sample in solution as the fluocolumbate with 2 grams of zinc added in small portions. For accurate work the standard cannot be diluted to match the sample. Since the standard is not stable, prepare several nonpermanent standards at the same time as the sample and compare by the series of standards method.

Miscellaneous

The reaction of columbium with hydroquinone to give a red color has been used for its colorimetric estimation.[11] The reaction is one in strong sulfuric acid and titanium and tungsten give the same color reaction.

[10] E. Meimberg, *Z. angew. Chem.* **26**, 83 (1913).

[11] Charles M. Johnson, *Iron Age* **157**, No. 14, 66-9, No. 15, 66-8 (1946).

CHAPTER 29

TANTALUM

TANTALUM plays a role similar to that of columbium in stainless steel by preventing grain disintegration. As a relatively minor metal it is not surprising that only one method is available.

SAMPLES

Minerals. Fuse a 2.0-gram sample with 4 grams of sodium pyrosulfate, cool, and leach with a saturated solution of ammonium oxalate. Transfer quantitatively to a volumetric flask and dilute to volume for use of aliquots.

STANDARD

Fuse 0.25 gram of tantalum pentoxide with 1 gram of sodium pyrosulfate, and cool. Extract with ammonium oxalate solution which has been acidified with 1-2 ml. of concentrated sulfuric acid. Dilute volumetrically to 100 ml. This is equivalent to 0.25 mg. of tantalum pentoxide per ml.

TANTALUM BY PYROGALLOL

In acid solution, or in 3 per cent ammonium oxalate solution, tantalum may be determined [1] by measurement of the yellow color produced by pyrogallol. Columbium does not interfere but, if titanium is present, determine it independently by the hydrogen peroxide method and apply a correction to the tantalum determination. The method detects 0.05-0.07 mg. of tantalum oxide in 1 ml. of solution.[2]

Procedure. If columbium has been determined in the solution with pyrogallol in the presence of sodium sulfite (page 498) add 1:10 sulfuric acid to 25 ml. of the solution until definitely acid and dilute to 50 ml. The yellow color present is due to tantalum. Determine by balancing

[1] G. Thanheiser, *Mitt. Kaiser-Wilhelm-Inst. Eisenforsch. Düsseldorf* **22**, 255-65 (1940); N. A. Vinogradova and E. I. Gushtyuk, *Zavodskaya Lab.* **11**, 223-6 (1945).

[2] N. F. Krivoshlykov, *Trudy LKKhTI* **1939**, No. 7, 103-22.

against a standard similarly prepared. Artificial standards prepared from ammonium chromate are also suitable. If titanium is present in moderate amount, subtract a correction, as it gives the same color.

If columbium has not been determined, make the aliquot of sample definitely acid with 1:10 sulfuric acid. Add an equal volume of 5 per cent pyrogallol solution saturated with sodium sulfite and dilute to 50 ml. Read the yellow color against natural or artificial standards. If small amounts of titanium are present, apply a correction. Omission of the sodium sulfite from the reagent is permissible.

CHAPTER 30

GOLD

GOLD IS SECOND in economic importance only to iron, and numerous methods, with varying degrees of reliability, have been developed for its colorimetric determination. Many are based on the reduction of the metal with a suitable reagent to its colloidal state. The desirable concentrations will give a red, purple, or blue color, but in lesser concentrations or in the presence of undue acidity, the yellow or pink is estimated. The color developed is greatly affected by the electrolyte concentration in the sample, which must therefore be matched in the standard.

SAMPLES

Antimony.[1] Dissolve a 10-gram sample in 30 ml. of concentrated hydrochloric acid and 10 ml. of concentrated nitric acid by heating to boiling. Evaporate to drive off the bulk of the nitric acid and take up with 20 ml. of concentrated hydrochloric acid. Add 10 ml. of saturated mercuric chloride solution and mix well. Add a concentrated solution of stannous chloride in concentrated hydrochloric acid dropwise with stirring until further additions fail to precipitate more mercury. The precipitate carries down the gold as amalgam.

Let the solution stand for 24 hours to insure complete precipitation. Filter and wash on the filter with 1:10 hydrochloric acid. Finally wash with water. Dry the filter and ash. Take up the residue in 3 drops of concentrated hydrochloric acid and a drop of concentrated nitric acid. Evaporate nearly to dryness, then take up in 3 drops of 1:20 nitric acid. Add water to about 5 ml. and filter the precipitate of silver chloride. Wash the precipitate with water and dilute to about 10 ml. This is designed for development with *p*-dimethylaminobenzalrhodanine.

Platinum Group Metals.[2] Dissolve 0.5 gram of sample in 6 ml. of concentrated hydrochloric acid and 2 ml. of concentrated nitric acid. Add 5 ml. of 10 per cent sodium chloride solution and evaporate to dry-

[1] N. S. Poluektov, *Trudy Vsesoyuz. Konferentsii Anal. Khim.* **2**, 393-8 (1943).
[2] F. E. Beamish, J. J. Russell and J. Seath, *Ind. Eng. Chem., Anal. Ed.* **9**, 174-6 (1937).

ness. Cool, moisten with three 1-ml. portions of 1:2 hydrochloric acid, and evaporate after each addition. Finally cool and dissolve the residue in 20 ml. of 1:3 hydrochloric acid. Filter and wash the precipitate. Adjust the volume of the filtrate and washings to a suitable volume, according to the gold content, and use an aliquot.

A detailed separation (page 515) includes isolation of gold in a solution suitable for use as sample.

Ores Poor in Gold. Moisten 100 grams of ore slightly but evenly in a glass-stoppered bottle with 1-2 ml. of equal volumes of bromine and ether. Shake at frequent intervals for 2 hours, during which time the interior of the bottle must be filled with bromine vapor. Add 50 ml. of water to the bottle and allow to stand for 2 hours, shaking occasionally. Filter and evaporate the filtrate to 20 per cent of its former volume. Add 5 ml. of saturated bromine-water and boil off excess bromine. Cool and dilute to 50 ml. for the use of all or an aliquot.

Cyanide Solutions.[3] Transfer 1 liter of cyanide solution containing gold to a beaker. Add 0.12 ml. of saturated lead acetate solution and stir until the precipitate dissolves. Add 0.75 gram of zinc dust and stir. Add 20 ml. of saturated sodium cyanide solution and 20 ml. of concentrated ammonium hydroxide. Stir vigorously for 5 minutes. Allow to settle and decant.

Transfer the lead sponge precipitate to a filter paper and wash the filter thoroughly with water. Dissolve the precipitate, except the gold, in 1:4 nitric acid solution, added cautiously. Wash the black gold particles that remain on the filter paper into the apex of the funnel with water. Discard the filtrate and washings. Add 10 ml. of saturated bromine water, 1 ml. at a time, over a period of 15 minutes. Keep the funnel covered to prevent bromine from escaping. Any remaining black particles on the filter paper are presumed to be carbon. Wash the paper with 8 ml. of water and heat the filtrate to 45° on a hot plate. Allow the solution to stand for 40 minutes to permit bromine to escape. Dilute to an appropriate volume and use as aliquot, preferably by stannous chloride reduction.

Alternatively, to 100 ml. of solution add about a gram of sodium peroxide. Boil for 2 minutes to destroy cyanides. To insure excess peroxide add 2 drops of 10 per cent lead acetate solution. A brown precipitate of lead dioxide forms and redissolves if excess peroxide is

[3] Colin G. Fink and Garth L. Putnam, *ibid.* 14, 468-70 (1942).

present. If necessary add more peroxide. Remove the flame and add about 0.1 gram of aluminum powder. Stir until evolution of hydrogen ceases. A black precipitate of gold and lead is obtained. Filter through a small paper.

Warm a mixture of 4 ml. of concentrated nitric acid and 6 ml. of concentrated hydrochloric acid. Pour through the paper several times until the precipitate is dissolved. The color may be developed in this solution or it may be diluted to a known volume for the use of aliquots. The smallest amount of lead possible should be used so that the precipitate to be redissolved will be small.

Whatever the method of separation may be, to estimate gold in cyanide solutions the cyanide must be removed before reduction. This may be done by oxidation with sodium peroxide or with bromine, by combination with another ion, or by volatilization, as well as by the precipitation methods cited.

Water and Aqueous Solutions.[4] Fill a 2-liter bottle with water to be analyzed. Add 5 grams of magnesium, then 50 ml. of a 2.7 per cent aqueous solution of mercuric chloride. Follow this addition with 50 ml. of concentrated hydrochloric acid and mix well. The reaction causes adequate stirring.

A fine gray precipitate of mercury and mercurous chloride settles in a few hours and carries with it the gold and silver content. Decant the main portion of the supernatant liquor, filter, and wash the precipitate. Dissolve the mercury from the paper with hot 1:4 nitric acid, leaving only the gold. Complete as for cyanide solutions (page 503) starting at "Wash the black gold particles. . . ."

Tissue.[5] Digest with nitric and sulfuric acids, with or without addition of hydrogen peroxide, according to the usual wet-ashing methods. Evaporate excess acid, including sulfuric acid, to dryness. Dissolve the residue, which contains the gold as metal, in 0.5 ml. of concentrated hydrochloric acid and 1.5 ml. of concentrated nitric acid. Transfer to a volumetric flask and dilute to such volume that 0.02-0.2 mg. of gold is present per ml. Use dimethylaminobenzalrhodanine as the developing agent.

Blood Plasma.[6] Transfer 1-5 ml. of sample to a calibrated 15-ml.

[4] William E. Caldwell and Kenneth N. McLeod, *ibid.* 9, 530-2 (1937).
[5] B. K. Merejkovsky, *Bull. soc. chim. biol.* 15, 1336-8 (1933).
[6] Walter D. Block and Oliver H. Buchanan, *J. Biol. Chem.* 136, 379-85 (1940).

Kjeldahl flask. Add 1 ml. of concentrated sulfuric acid. Introduce a glass bead and 0.2 ml. of caprylic alcohol, and digest slowly over a low flame until the solution begins to char. Continue heating for 7 minutes and cool. To oxidize the organic matter, add 30 per cent hydrogen peroxide dropwise until the solution is water clear. Evaporate completely to dryness. Cool and add 0.5 ml. of a 3:1 mixture of concentrated hydrochloric and nitric acids. Dilute to 15 ml. and use the entire sample as aliquot, preferably estimating by the use of *o*-dianisidine.

Urine.[7] Transfer a 10-50 ml. sample to a 100-ml. calibrated Kjeldahl flask. Add 3-5 ml. of concentrated sulfuric acid. Introduce a glass bead and 0.5 ml. of caprylic alcohol and digest slowly over a low flame until the solution begins to char. Continue heating for 7 minutes and cool. Add 30 per cent hydrogen peroxide dropwise to oxidize the organic matter, until the solution is water clear. Evaporate completely to dryness, cool, and add 1.5 ml. of concentrated hydrochloric acid and 0.5 ml. of concentrated nitric acid. Keep the flask 5-6 cm. above the flame to prevent decomposition of gold chloride. Evaporate to 0.2-0.3 ml. to remove the acids which might interfere with the color produced. Cool somewhat and pass in a slow stream of air to evaporate the remaining acid. Cool to room temperature and dilute to 75 ml., using the entire sample as aliquot, estimating the gold content preferably by the o-dianisidine method.

Isolation. Measure out an aliquot of sample containing 0.001-0.01 mg. of gold. Adjust the acidity to about 1:10 with hydrochloric acid. Add 0.5 ml. of a 0.05 per cent solution of tellurium oxide in 1:10 hydrochloric acid. Mix well and add 2 ml. of 10 per cent stannous chloride solution in 1:10 hydrochloric acid. This reduces tellurium and gold, but does not reduce iron, copper, and various other metals to metallic form. In some cases the amount will require increase. A brown colloidal dispersion of tellurium is produced. This coagulates on heating to boiling for 5-10 minutes and carries down the gold with it. Filter on an inorganic filter and wash with 1:20 hydrochloric acid.

Dissolve the residue from the filter with a hot mixture of 1 ml. of concentrated nitric acid and 2 ml. of concentrated hydrochloric acid. Wash the filter with hot water and evaporate the filtrate just to dryness on a steam bath. Take up the residue in a drop of 1:1 hydrochloric acid and add water. This is designed for determination by *p*-diethylaminobenzalrhodanine.

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[7] *Ibid.*

Standards

Transfer 0.1539 gram of auric chloride to a 1-liter flask. Dissolve in water and dilute to volume. Each ml. of this solution is equivalent to 0.1 mg. of gold. The standard is stable for 2-3 days.

Alternatively, dissolve 3.11 mg. of pure gold in a mixture of 7 ml. of concentrated hydrochloric acid and 3 ml. of concentrated nitric acid, and dilute to 1 liter. This corresponds to 48 grains of gold per ton of solution. Keep in a dark bottle. If results are to be reported in ppm., use 5 mg. of gold; the final solution contains 5 ppm.

Gold as Colloidal Gold

A small amount of gold salt in solution may be determined by reduction to the colloidal state. Colloidal gold may exist in the yellow form in solutions of low acidity and in the purple form in solutions of high acidity.[8] The former is stable, but the latter may precipitate in a few hours. There are other variations depending on electrolyte content and reducing agent used. Such a colloidal solution of gold in the finest degree of dispersion is red. This is changed toward a blue modification of larger particle size known as "Purple of Cassius," by the presence of a small amount of electrolyte. The blue suspension separates as a gelatinous mass on standing.

In the reduction of gold solutions by formaldehyde, benzidine, α-naphthylamine, stannous chloride, or mercurous chloride, the presence of heavy metals and alkali metals influences the results.[9]

The concentration [10] of the acid in the colorimetric medium is an important factor in the color produced by stannous chloride. When sufficient hydrochloric acid is added to the yellow form of colloidal gold to make the solution 1:5-1:1, the color changes to an unstable blue or purple. The addition of hydrochloric acid to solutions containing 2-20 mg. of gold per liter causes the yellow acid solution to become colorless for a brief period before the blue or purple color develops. Under controlled conditions, the color ranges from light yellow to light brown, so that a constant tint is obtained regardless of the gold concentration. These factors are brought out in Table 7.

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[8] Colin G. Fink and Garth L. Putnum, *Ind. Eng. Chem., Anal. Ed.* 14, 468-70 (1942).

[9] I. N. Plaksin and N. A. Suvorovskaya, *Zavodskaya Lab.* 7, 1202-3 (1938).

[10] Colin G. Fink and Garth L. Putnam, *Ind. Eng. Chem., Anal. Ed.* 14, 468-70 (1942).

As the concentration increases, the length of time required for the color to develop increases. Particularly if time is limited, the test is more sensitive in weakly acid solutions. In dilute acid solution, the color intensity is not a function of the concentration of the stannous chloride reagent. The concentration of oxidizing agents, which tend to form more readily hydrolyzable stannic compounds, and of salts, which accelerate clouding of the stannous chloride solutions, should be kept at a minimum.

Table 7. Effect of Acid Concentration on Tint

Total HCl Concentration in Developed Solution N	*Tint after Standing for 15 Minutes*
0.002	Yellow
0.08	Light brown
0.16	Tan with faint purple tinge
0.32	Tan with distinct purple tinge
0.64	Purple

Effect of Acid Concentration on Velocity of Color Development

Total HCl Concentration in Developed Solution N	*Seconds after Start of Second Trial, Required for Colors to Match*	*Tint after 20-30 Minutes' Standing*
0.002	32	Yellow
0.08	73	Yellow
0.16	400	Light brown
0.64	820	Purple
5.0	1420	Dark blue

Procedure. *Reduction with Stannous Chloride.* Prepare a stannous chloride solution by heating together 11.3 grams of stannous chloride and 1.17 ml of concentrated hydrochloric acid. Keep at the boiling point for 2-3 minutes and cool the clear solution to 40-50°. Pour into a 200-ml. volumetric flask containing about 150 ml. of water, then dilute to volume. Keep 1-2 grams of tin in the bottle to insure its remaining in a reduced condition.

Dilute an aliquot of sample containing 0.02-0.2 mg. of gold in known dilute acid solution to about 40 ml. At the same time prepare one or more standards of similar acidity and volume. To each add 2 ml. of prepared stannous chloride solution and dilute to 50 ml. Close with a rubber stopper pretreated with chlorine water and allow to stand for 10 minutes. Dilution or balancing are suitable if the acidity is in a range which gives a yellow sol. For blue, red, and purple sols it is necessary to

use a series of standards. The transmittance may also be read without a filter provided the type of color developed in the sample is similar to that in the standard.

Reduction with Benzidine.[11] Evaporate an amount of platinum- and iron-free sample containing 0.2-2 mg. of gold nearly to dryness to eliminate excess acid. Transfer to a volumetric flask with about 30 ml. of water. Dissolve 1 gram of benzidine in 50 ml. of 1:10 hydrochloric acid. Add 1 ml. of this to the sample and immediately dilute to volume. Let stand for 2 hours and either compare with standards developed at the same time or read the transmittance around 500 mμ. The color developed does not conform to Beer's law.

Reduction with Glucose.[12] Evaporate an appropriate sample just to dryness to drive off any acid present, then take up in water. Similarly provide a comparable standard. Dilute each nearly to 50 ml. and add 0.25 ml. of 5 per cent glucose solution to each. Heat to 90° and add slowly, with shaking, 0.20-0.25 ml. of a 5 per cent solution of anhydrous potassium carbonate. Compare the intensity of the red color developed. Alternatively, read the transmittance of the solution and compare with a suitable calibration curve. The latter method is of doubtful reliability.

Reduction with α-Naphthylamine Hydrochloride.[13] The addition of naphthylamine hydrochloride to a dilute acid solution of gold results in a violet color. The color obeys Beer's law and is accurate to 2 per cent. Palladium interferes, but copper, zinc, lead and iron do not.

Dilute a suitable aliquot nearly to 9 ml., add 1 ml. of 0.1 per cent solution of α-naphthylamine hydrochloride, and dilute to 10 ml. Compare the color with that of similarly treated standards. The color increases in the first 2-3 minutes, then remains constant for 1-2 hours.

Reduction with Metaphenylene Diamine. To a 50-ml. sample add 5 ml. of 0.5 per cent solution of metaphenylene diamine. Add 5 ml. of 1:1 sulfuric acid. A yellow to dark brown color is formed, dependent on the strength of the gold solution. A solution as dilute as 0.005 per cent will show a color by this method. If the reagent becomes pink on standing in the light, decolorize with activated carbon.

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[11] Jenő Plank, *Magyar Chem. Folyóirat* **47**, 85-90 (1941).

[12] Leonor S. V. de Bollini, *Rev. facultad cienc. quím.* (Univ. nacl. La Plata) **16**, 103-8 (1941).

[13] I. A. Paul'sen and S. M. Pevzner, *J. Applied Chem.* (U.S.S.R.) **11**, 697-700 (1938).

Reduction with Phenylhydrazine. To the sample solution add 1 gram of citric acid and 5 drops of a 10 per cent solntion of phenylhydrazine hydrochloride. A blue-violet color is obtained by transmitted light. This will detect one part in 2 million. Comparison with a series of standards is advisable. Such standards keep several hours, but eventually give a black deposit.

Reduction with Phosphine.[14] Moisten filter paper with a solution of sample in slightly acid solution. Place in one arm of a U-tube, and in the other arm place a strip of filter paper which has heen moistened with a similarly acid standard solution of known gold content. Into the flask attached to the U-tube transfer a quantity of phosphonium iodide and introduce potassium hydroxide dropwise to produce phosphine. Expose the contents of the U-tube to the phosphine, moisten the filter papers with 1:10 hydrochloric acid, and compare sample and standard.

Reduction with Formaldehyde.[15] To 50 ml. of sample add sufficient 20 per cent sodium hydroxide solution for neutralization and 1 ml. in excess On addition of 1 ml. of 40 per cent formaldehyde a blue color is developed which may be balanced against a standard. The optimum concentration for this determination is 1 part in 40,000. Variations of this method include the addition of a small amount of amino acid to the formaldehyde-alkali mixture.[16] This reducing agent colors the sol red-violet. The reagent is not applicable in the presence of platinum, palladium, mercury, silver, arsenic, tin, tungsten, molybdenum, and vanadium. Lead, aluminum, or zinc in moderate amonnts do not interfere.

Gold by *o*-Dianisidine

Chlorauric acid forms an intense red color in slightly acid solution with *o*-dianisidine,[17] and addition of hydroquinone solution causes precipitation of gold and consequent decolorization.[18] The reaction takes place in a slightly acid solution buffered with potassium bifluoride to prevent any ferric ions present from reacting with the reagent. Preliminary precipitation hy using a 1:10 hydrochloric acid solution of telluric acid saturated with sulfur dioxide serves to isolate gold in solu-

[14] N. D. Costeanu, *Bull. soc. chim.* [5] 3, 1527-30 (1936).
[15] A. Muller and A. Foix, *Bull. soc. chim.* 31, 717-20 (1922).
[16] Tadao Itô, *J. Chem. Soc. Japan* 58, 288-91 (1937).
[17] W. D. Block and O. H. Buchanan, *J. Biol. Chem* 136, 379-85 (1940); *J. Lab. Clin. Med.* 28, 118-20 (1942).
[18] W. B. Pollard, *Analyst* 62, 597-603 (1937).

tion without any inorganic salts which might interfere. Free halogens interfere.

Procedure. To an aliquot of sample of about 75 ml., add 0.75 ml. of 1:4 hydrochloric acid, 8 ml. of 10 per cent potassium fluoride, and 1 ml. of 0.025 per cent solution of *o*-dianisidine in 1:250 hydrochloric acid. If the sample is blood plasma, 0.75 per cent of potassium bifluoride replaces the potassium fluoride. For smaller aliquots reduce the amounts of reagent. Dilute volumetrically to 100 ml. and read photometrically within 3-10 minutes at the maximum intensity. Stopper to prevent color development by the reagents in the presence of air.

Gold by *p*-Dimethylaminobenzalrhodanine

When treated with the reagent, gold gives a red to violet color of the compound formed.[19] This is not unlike that due to colloidal gold. The limit of determination is about 2 ml. containing 2 ppm. The same color is developed from palladium, platinum, mercury, and cuprous ions. The solution may be saturated with silver chloride without interference. All electrolytes reduce the sensitivity. Color development continues for about 20 minutes after mixing. The reagent itself gives a yellow color.

Procedure. Prepare a fresh 0.0006 per cent solution of *p*-dimethylaminobenzalrhodanine by adding 1 ml. of 0.03 per cent solution in ethanol to 13 ml. of benzene and diluting to 50 ml. with chloroform. Measure out an aliquot of sample solution containing 0.0002-0.002 mg. of gold. Evaporate nearly to dryness if, as is usually the case, it contains excess acid. Take up in water and dilute to about 5 ml. Add 3 drops of concentrated nitric acid, then 0.5-1.0 ml. of the yellow reagent. Shake the solution vigorously. In the presence of gold, the chloroform-benzene layer will turn from yellow to pink-violet. Compare with similarly prepared standards.

Alternatively, adjust the solution to approximately 1:100 with hydrochloric acid and a volume of 20 ml. Add 2 drops of a 0.1 per cent solution of the reagent in ethanol and dilute to 25 ml. After 20 minutes read the transmittance of the solution so developed using a green filter.

Gold by Mercurous Chloride

In the absence of interfering metals, the effect of gold in coloring mercurous chloride may be used for its colorimetric estimation.[20] The

[19] N. S. Poluektov, *Trudy Vsesoyuz, Konferentsii Anal. Khim.* **2,** 393-8 (1943).

[20] Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* **6,** 437-9 (1934); *ibid.* **11,** 86-8 (1939).

color is due to the reducing action of mercurous chloride. Arsenic, platinum, palladium, selenium, tellurium, and iodine, which would ordinarily coprecipitate, may be prevented from interfering by controlling the conditions of precipitation. Nitrates, per salts, free halides, hypophosphites, and large quantities of cupric and ferric salts interfere because of their oxidizing action. Color variations depend on the quantity of metal precipitated, on the temperature, solution purity, and to some extent on the solution concentration of the element before precipitation. The use of standards run under identical conditions reduces these errors. Colored solutions do not interfere and the reaction is stated to be more sensitive than the others in use. Since it depends on the colloidal color, it is hardly likely to be more sensitive, but it is applicable in cases where the other methods are not. Over 0.003 gram of copper or 0.006 gram of iron will interfere.

Procedure. If palladium, platinum, selenium, tellurium, and arsenic are present, add a 1 per cent oxalic acid solution to a solution of these metals in 1:50 hydrochloric acid until precipitation is complete. Boil and filter the gold precipitate. At this point, care should be exercised because the gold precipitate is sometimes very fine; this makes filtration difficult and tends to occlude palladium.[21] Save the filtrate for determination of palladium, platinum, selenium, tellurium, and arsenic. Dissolve the precipitate in 1:50 hydrochloric acid saturated with chlorine. Boil to remove chlorine. If none of the above metals is present, the treatment with oxalic acid may be eliminated.

Adjust the temperature to 45° and transfer 5 ml. of sample to a colorimeter tube. Add 0.1 gram of mercuric chloride followed by an equal weight of mercurous chloride, mix, allow to settle, and compare with similarly prepared standards. The colors produced are the following:

Gold in mg.	*Color on Mercurous Chloride*
0.20	Dark Purple
0.10	Pinkish purple
0.05	Purplish pink
0.02	Strong pink
0 002	Light pink
0 0002	Very light pink
0 00005	Faint coloration

[21] F. E. Beamish, J. J. Russell and J. Seath, *Ibid.* 9, 174 6 (1937).

Mercuric chloride may be effectively employed by reducing in hydrochloric acid solution with 1 gram of magnesium per liter of sample.[22]

Miscellaneous

Auric ion in solution may be detected with a chloroform extract of the colorless leuco compound of *o*-nitrobrilliant green, $O_2NC_6H_4CH\cdot(C_6H_4N(C_2H_5)_2)_2$, in acetic acid.[23] The reaction is one of oxidation of the leuco base to the dyestuff with reduction of auric to aurous ion. It follows that oxidizing agents will interfere. The reaction is sensitive to 0.05 ppm. The leuco compound of malachite green, $C_6H_5CH(C_6H_4N(CH_3)_2)_2$ will similarly detect 0.3 mg. of gold per liter. As reagent dissolve 0.05 gram of the base in 5 ml. of 95 per cent ethanol. Add 0.5 ml. of 80 per cent acetic acid and boil for 3-5 minutes. Add 10 ml. of ethanol and 35 ml. of acetic acid-sodium acetate buffer for pH 3.6. For determination add 1 ml. of the reagent to sample and standard solutions, dilute to known volumes, boil for 2 minutes, and cool. Balancing is suitable and the use of a yellow filter adds to the sensitivity.

In dilute hydrochloric acid solution, the addition of 1 ml. of a 0.1 per cent solution of o-tolidine in 1:10 hydrochloric acid solution produces a sensitive reaction with chlorauric acid.[24] One ppm. of gold gives a bright yellow, and 0.1 ppm. gives a yellow color detectable in a depth of 10 cm. Ferric iron, ruthenium, osmic acid, vanadates, tungstates, nitrous acid and free chlorine must be absent. If copper is present and gives a green color, tint the standard with copper to match before adding the reagent to either. The color is fully developed in 5 minutes and fades slowly after one-half hour.

Bivalent gold reacts with dithizone to give a yellow color.[25] The applicability to quantitative estimation has not been fully explored.

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[22] William E. Caldwell and Kenneth N. McLeod, *ibid.* 9, 530-2 (1937).
[23] L. M. Kul'berg, *Zavodskaya Lab.* 5, 170-5 (1936).
[24] W. B. Pollard, *Analyst* 44, 94-5 (1919).
[25] Hellmut Fischer, *Angew. Chem.* 47, 685-92 (1934); *ibid.* 50, 919-32 (1937).

CHAPTER 31

PLATINUM

PLATINUM is so noble that it seldom occurs other than as the metal, but an arsenide is associated with nickel deposits. It is commonly alloyed with other metals of the platinum series. Amounts too small to determine by assay are customarily estimated colorimetrically. The principle methods are as the iodide or reduced to the platinous chloride by means of stannous chloride. The methods are equally accurate.

SAMPLES

Mixed Platinum Metals.[1] By this procedure the elements are separated in the following order: Osmium, ruthenium, platinum, palladium, rhodium, iridium. While the separation is designed for gravimetric use it is applicable to smaller amounts which will necessarily be estimated colorimetrically. Accuracy within 0.1 mg. is obtained on 100-300 mg. quantities and can be improved by micro manipulation. Because of the variability in size of sample, details of the exact amounts of reagents are not given.

Separation of Osmium. Acidify the sample so that it contains 10 per cent of nitric acid by volume. Transfer to a 700-ml. distilling flask having inlet and delivery tubes sealed in and connected to a set of three 300-ml. absorbing flasks by ground-glass joints. Depending on the amount of sample, the size of this equipment may be proportionately reduced. Fill the absorbing bottles with 1:1 hydrochloric acid saturated with sulfur dioxide. Heat the sample solution to boiling and pass a gentle current of air through it until the osmium tetroxide is completely carried over. Combine the absorbing solutions and evaporate to a sirup to recover the osmium in the sample for further treatment.

Separation of Ruthenium. Evaporate the osmium-free residue from the flask on a steam bath to eliminate nitric acid. Add a few ml. of concentrated hydrochloric acid and evaporate to remove oxides of

[1] Raleigh Gilchrist, *Technical News Bulletin of the National Bureau of Standards*, June, 1935, 62 3.

nitrogen. Repeat several times. Add 10 ml. of concentrated sulfuric acid and heat until sulfur trioxide fumes are evolved. Return the solution and any metallic platinum to the distilling flask and dilute to about 100 ml. Add sodium bromate in excess and distill as before, absorbing the distilled ruthenium tetroxide in 1:1 hydrochloric acid saturated with sulfur dioxide. To recover the ruthenium, concentrate the absorbing solution as described for osmium.

Separation of Platinum. Transfer the solution from the distilling flask and neutralize to pH 6.0 with 1:1 ammonium hydroxide. Add sodium bromate to the solution and heat to boiling to precipitate the hydrated oxides of palladium, iridium, and rhodium. Filter, redissolve the precipitate in 1:1 hydrochloric acid, and reprecipitate the hydrated oxides to recover sorbed platinum. Save the oxides for further treatment and combine the platinum filtrates. Acidify and add hydrogen peroxide to destroy excess bromate. Boil to decompose excess hydrogen peroxide and use all or an aliquot.

Separation of Palladium. Dissolve the mixed hydrated oxides in 1:1 hydrochloric acid and dilute with water to a reasonable acidity. Add a solution of dimethylglyoxime in excess to precipitate the palladium. Filter and ignite the precipitate to decompose the glyoxime. Dissolve the metal or oxide in a 1:3 mixture of concentrated nitric and hydrochloric acids and evaporate just to dryness. Take up the residue in 1:1 nitric acid and use all or an aliquot.

Separation of Rhodium. Add 10 ml. of concentrated sulfuric acid to the filtrate from palladium glyoxime. Evaporate to a small volume and destroy excess of dimethylglyoxime by small volumes of concentrated nitric acid. Heat to fumes of sulfur trioxide to drive off nitric acid. Dilute to a suitable volume, such as 200 ml., and heat to boiling. Add a slight excess of titanous chloride to precipitate metallic rhodium. Filter and dissolve the precipitated metal in a small volume of boiling 1:1 sulfuric acid. Dilute to a suitable volume and again precipitate rhodium. Filter and dissolve the residue in the minimum possible volume of hot 1:1 sulfuric acid. Use this solution as sample.

Separation of Iridium. Adjust the combined filtrates containing iridium to pH 6. Add an excess of cupferron solution. Filter and boil to precipitate the hydrated oxide of iridium. If interference by the excess of cupferron occurs, evaporate to dryness, destroy the organic

matter by heating with nitric acid, and again take up to a suitable volume, adjusting to pH 6.

Dissolve the precipitate of hydrated iridium oxide in a 1:3 mixture of concentrated nitric and hydrochloric acids and evaporate just to dryness. Add a few drops of concentrated hydrochloric acid and again evaporate just to dryness. Take up with distilled water and use as sample.

Separation of Gold, Platinum, and Palladium. Add 1 gram of oxalic acid for every 20 ml. of sample solution and boil. Gold is precipitated. Filter on fine paper and wash. Dissolve the precipitate from the paper and beaker with 1:10 hydrochloric acid containing free chlorine. Boil to drive off free chlorine and use as sample for determination of gold (Chapter 30).

Acidify the filtrate containing platinum and palladium with a few drops of concentrated sulfuric acid, and evaporate nearly to dryness to decompose oxalic acid. If the residue is not completely soluble in water acidify with 1:10 hydrochloric acid containing free chlorine. In that case boil to dissolve, evaporate to dryness on a water bath, and take up with water. The final acidity must not exceed 4 per cent as hydrochloric acid. Add 5 per cent by weight of crystallized copper sulfate and use for estimation of palladium and platinum, preferably by reduction by stannous chloride.

Filings and Sweeps. Dissolve a suitable sample according to concentration in a mixture of 3 ml. of concentrated nitric acid and 9 ml. of concentrated hydrochloric acid. Evaporate to dryness and take up with 10 ml. of 1:5 hydrochloric acid. Add 1 gram of 20-mesh zinc and warm if necessary to hasten the action.

Filter off the precipitated metals, wash, and dissolve all except gold and platinum with cold 1:5 nitric acid. Filter off the gold and platinum. The removal of copper, always found in such materials as jewelers' filings, is important as substantial amounts will affect the final color. Wash the deposit of gold and platinum thoroughly to remove all the copper nitrate. Dissolve the gold and platinum in a mixture of 3 ml. of concentrated nitric acid and 9 ml. of concentrated hydrochloric acid, and evaporate to dryness. Take up with 10 ml. of 1:20 hydrochloric acid.

Prepare a ferrous sulfate solution by dissolving 2 grams in 100 ml. of 1:20 hydrochloric acid Add 2 ml. of this solution, free from ferric ions, to the platinum and gold solution Ferric ion will cause a yellow color. Warm to 40°, but not higher, and maintain at that temperature

nitrogen. Repeat several times. Add 10 ml. of concentrated sulfuric acid and heat until sulfur trioxide fumes are evolved. Return the solution and any metallic platinum to the distilling flask and dilute to about 100 ml. Add sodium bromate in excess and distill as before, absorbing the distilled ruthenium tetroxide in 1:1 hydrochloric acid saturated with sulfur dioxide. To recover the ruthenium, concentrate the absorbing solution as described for osmium.

Separation of Platinum. Transfer the solution from the distilling flask and neutralize to pH 6.0 with 1:1 ammonium hydroxide. Add sodium bromate to the solution and heat to boiling to precipitate the hydrated oxides of palladium, iridium, and rhodium. Filter, redissolve the precipitate in 1:1 hydrochloric acid, and reprecipitate the hydrated oxides to recover sorbed platinum. Save the oxides for further treatment and combine the platinum filtrates. Acidify and add hydrogen peroxide to destroy excess bromate. Boil to decompose excess hydrogen peroxide and use all or an aliquot.

Separation of Palladium. Dissolve the mixed hydrated oxides in 1:1 hydrochloric acid and dilute with water to a reasonable acidity. Add a solution of dimethylglyoxime in excess to precipitate the palladium. Filter and ignite the precipitate to decompose the glyoxime. Dissolve the metal or oxide in a 1:3 mixture of concentrated nitric and hydrochloric acids and evaporate just to dryness. Take up the residue in 1:1 nitric acid and use all or an aliquot.

Separation of Rhodium. Add 10 ml. of concentrated sulfuric acid to the filtrate from palladium glyoxime. Evaporate to a small volume and destroy excess of dimethylglyoxime by small volumes of concentrated nitric acid. Heat to fumes of sulfur trioxide to drive off nitric acid. Dilute to a suitable volume, such as 200 ml., and heat to boiling. Add a slight excess of titanous chloride to precipitate metallic rhodium. Filter and dissolve the precipitated metal in a small volume of boiling 1:1 sulfuric acid. Dilute to a suitable volume and again precipitate rhodium. Filter and dissolve the residue in the minimum possible volume of hot 1:1 sulfuric acid. Use this solution as sample.

Separation of Iridium. Adjust the combined filtrates containing iridium to pH 6. Add an excess of cupferron solution. Filter and boil to precipitate the hydrated oxide of iridium. If interference by the excess of cupferron occurs, evaporate to dryness, destroy the organic

matter by heating with nitric acid, and again take up to a suitable volume, adjusting to pH 6.

Dissolve the precipitate of hydrated iridium oxide in a 1:3 mixture of concentrated nitric and hydrochloric acids and evaporate just to dryness. Add a few drops of concentrated hydrochloric acid and again evaporate just to dryness. Take up with distilled water and use as sample.

Separation of Gold, Platinum, and Palladium. Add 1 gram of oxalic acid for every 20 ml. of sample solution and boil. Gold is precipitated. Filter on fine paper and wash. Dissolve the precipitate from the paper and beaker with 1:10 hydrochloric acid containing free chlorine. Boil to drive off free chlorine and use as sample for determination of gold (Chapter 30).

Acidify the filtrate containing platinum and palladium with a few drops of concentrated sulfuric acid, and evaporate nearly to dryness to decompose oxalic acid. If the residue is not completely soluble in water acidify with 1:10 hydrochloric acid containing free chlorine. In that case boil to dissolve, evaporate to dryness on a water bath, and take up with water. The final acidity must not exceed 4 per cent as hydrochloric acid. Add 5 per cent by weight of crystallized copper sulfate and use for estimation of palladium and platinum, preferably by reduction by stannous chloride.

Filings and Sweeps. Dissolve a suitable sample according to concentration in a mixture of 3 ml. of concentrated nitric acid and 9 ml. of concentrated hydrochloric acid. Evaporate to dryness and take up with 10 ml. of 1:5 hydrochloric acid. Add 1 gram of 20-mesh zinc and warm if necessary to hasten the action.

Filter off the precipitated metals, wash, and dissolve all except gold and platinum with cold 1:5 nitric acid. Filter off the gold and platinum. The removal of copper, always found in such materials as jewelers' filings, is important as substantial amounts will affect the final color. Wash the deposit of gold and platinum thoroughly to remove all the copper nitrate. Dissolve the gold and platinum in a mixture of 3 ml. of concentrated nitric acid and 9 ml. of concentrated hydrochloric acid, and evaporate to dryness. Take up with 10 ml. of 1:20 hydrochloric acid.

Prepare a ferrous sulfate solution by dissolving 2 grams in 100 ml. of 1:20 hydrochloric acid Add 2 ml. of this solution, free from ferric ions, to the platinum and gold solution. Ferric ion will cause a yellow color. Warm to 40°, but not higher, and maintain at that temperature

for 45 minutes. As an alternative it may be allowed to stand for 3-4 hours, but heating is preferable because it coagulates the precipitate. Not over 0.2 gram of ferrous sulfate should be present per mg. of gold. Gold is precipitated and carries with it a trace of platinum. The amount so carried down is very small. Filter and evaporate the filtrate just to dryness. Take up with hydrochloric acid of a concentration appropriate to the method of determination.

Metallic Sublimate.[2] Dissolve a 10-gram sample in the minimum amount of a 3:1 mixture of concentrated hydrochloric acid and concentrated nitric acid. Dilute to about 50 ml. and add 1 ml. of 0.75 per cent mercuric chloride solution. To this add 10 per cent stannous chloride solution, dropwise, until no further deposit of metallic mercury appears. Boil the solution for 5 minutes, let it stand to complete coagulation, and filter. Wash the filter with 1:10 hydrochloric acid, then with 1 per cent ammonium nitrate solution. Discard the filtrate and washings. Ash and let cool. Add about 0.25 gram of ammonium iodide and heat until no more fumes are given off. Repeat.

Take up the platinic iodide residue with 2 drops of concentrated nitric acid and 6 drops of concentrated hydrochloric acid. Drive off substantially all of the excess acid and take up with water to use for development by stannous chloride.

Ores, Sands, or Concentrates. Grind the sample to pass a 100-mesh screen, or if difficultly fusible substances such as chromite or zircon are present, grind to pass a 150-mesh screen. Mix one assay ton of the carefully sampled ore with a suitable flux. Table 8 of a number of charges gives typical examples. All charges are in grams.

The quantity of niter or argols is only approximate in each case and should be so varied as to give a 30-gram lead button. A borax glass cover layer is used for each.

If less than 15 times as much silver as platinum is present, add silver chloride or nitrate to make up the deficiency. Excess silver is required to render the platinum soluble in nitric acid when parting, to assist in removing the last traces of lead in cupelling, and to diminish loss in the cupel. For an unknown ore use 0.05 gram of silver chloride.

Flux as in the ordinary fire assay for gold and silver but after the fusion is quiet raise the temperature higher than usual and continue heating for an hour. Remove the crucible and let cool without agitating.

[2] N. S. Poluektov and F. G. Spivak, *Zavodskaya Lab.* 11, 398-403 (1945).

TABLE 8. CHARGES FOR SEPARATION OF PLATINUM BY FIRE ASSAYING

	Silicate	*Hematite*	*Limestone*	*Roasted Sulfide*	*Black sand, largely magnetic iron oxide*	*Chromite*	*Sulfides*	*Sulfides and Barite*
Ore	29.166	29.166	29.166	29.166	29.166	29.166	29.166	29.166
Sodium Carbonate	30-60	30	....	40	....	....	30-60	....
Sodium Bicarbonate	....	....	40	....	20	60	....	30
Borax	....	....	20	20	....	....	10	20
Borax Glass	5	8	....	....	30	20	....	....
Litharge	60-90	70	40	40	66	35	50-150	150
Silica	....	7	5	4	....	15	0-10	....
Fluospar	....	....	....	....	....	....	....	15
Argols	2.5	3.5	2.5	7	4	3	....	....
Niter	....	....	....	....	....	....	21	8
Sodium Chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Break the button free from slag and cupel at a high temperature. This causes such loss of silver that it must be separately determined.

When the platinum constitutes more than 1.6 per cent of the bead, the latter has a frosted appearance. Iridium causes a roughness of finer texture. Palladium gives the bead an embossed appearance. Ruthenium, if present in quantity, turns the surface a bluish black, leaving a black scum on the cupel. After cupelling, part the button with nitric acid, first with 1:4 acid, then 1:1, and finally 2:1. If gold, silver, and all the platinum metals are in the ore, silver, palladium, and platinum are dissolved by this treatment, leaving gold, iridium, rhodium, and some ruthenium and osmium. Most of the osmium and part of the ruthenium are oxidized and lost during cupellation. Part of the iridium may not collect in the silver and will be lost on the cupel. If considerable platinum is found some will remain undissolved. Filter the residue on an ashless paper, ignite and save for the recovery of platinum as well as for the determination of iridium and rhodium.

Add dilute hydrochloric acid to the filtrate slowly with constant stirring, to precipitate silver. Let stand over night, filter off the silver chloride and wash with 1:20 nitric acid. If the precipitate is pink redissolve in the minimum amount of 1:1 ammonium hydroxide and reprecipitate with 1:1 hydrochloric acid to recover occluded platinum or palladium.

Evaporate the filtrate just to dryness. Take up with 10 ml. of 1:5 hydrochloric acid and again evaporate just to dryness. Take up with 10 ml. of 1:5 hydrochloric acid, and evaporate nearly to dryness. When cold take up with 5 ml. of cold water, and filter to remove the last trace of silver. Make the filtrate slightly alkaline with sodium carbonate, add 1 ml. of 30 per cent formic acid, and boil in a covered beaker until all the platinum and palladium are precipitated. This will usually require about 30 minutes. Filter, wash with hot water, and ignite in an "Impervite" crucible. The platinum metals adhere to both glazed and unglazed porcelain. Add 1 ml. of 30 per cent formic acid to the filtrate and boil again to insure complete precipitation. If the first parting solution is yellow or orange, the presence of palladium is indicated. Warm the metals with 1:4 nitric acid, which dissolves the palladium, filter, wash, and ignite the platinum residue.

Treat the residue from the nitric acid parting, which may contain gold, iridium, rhodium, some ruthenium, osmium, and undissolved platinum, with 1.5 ml. of 1:5 nitric acid and 4.5 ml. of 1:5 hydrochloric acid, thus dissolving the gold and platinum. Filter, evaporate the acid

solution of gold and platinum to dryness, and take up with 5 ml. of 1:1 hydrochloric acid. Again evaporate to dryness, treat a second time with 5 ml. of 1:1 hydrochloric acid, and evaporate nearly to dryness. Take up with 5 ml. of cold water, and filter if necessary. Add 0.1 gram of oxalic acid and boil until the gold is all precipitated. It is best to let the solution stand overnight before filtering off the gold. After filtering, neutralize with 20 per cent sodium carbonate solution, boil with 1 ml. of 30 per cent formic acid, filter off the platinum, and ignite. Combine with the platinum previously separated.

If the total platinum amounts to more than 0.2 mg., it may be determined by weight. If less than 0.2 mg. of platinum is present, dissolve the ignited platinum in a mixture of 1.5 ml. of concentrated nitric acid and 4.5 ml. of concentrated hydrochloric acid, and evaporate just to dryness twice with hydrochloric acid. Extract with hydrochloric acid of a concentration appropriate to the method of color development to be used.

If the quantity of platinum is small, run several samples of an assay ton each, combine the lead buttons, scorify to about 30 grams and proceed as above. As a blank on the reagents add the approximate amounts of gold, silver, and platinum to the crucible charge and carry through the same procedure.

Standard

Clean a soft sheet of platinum with concentrated nitric acid, then with concentrated hydrochloric acid, and finally heat to a bright red. Dissolve 0.1000 gram of clean platinum in a 1:3 mixture of concentrated nitric and hydrochloric acids, evaporate nearly to dryness, and add 5-10 ml. of concentrated hydrochloric acid. Add 0.06 gram of sodium chloride and evaporate just to dryness on a water bath to remove excess acid. Take up with distilled water. A clear yellow solution of sodium chloroplatinate should be obtained. If the solution is turbid some decomposition has occurred. In that case evaporate to dryness, add concentrated hydrochloric acid or a 1:3 mixture of concentrated nitric and hydrochloric acids and proceed as before.

Dilute the solution containing 0.1 gram of platinum to 100 ml. Each ml. corresponds to 1 mg. of platinum. By diluting 50 ml. of this solution to 500 ml. a second standard is obtained which contains 0.1 mg. of platinum per ml. The latter tends to form deposits after a few weeks. This may be prevented by adding 5 ml. of concentrated hydrochloric acid.

Platinum as the Iodide

Platinum is determined by changing the platinic chloride to platinic iodide which dissolves in excess potassium iodide solution to give a rose to red color of an iodoplatinate.[3] The color develops slowly. Sulfites, thiosulfates, ammonium hydroxide, and mercuric chloride destroy the color of the iodide. Palladium develops the same color in about the same time and rhodium develops it even more slowly. Gold, iron, copper, and bismuth must be absent because of the various reactions they give with iodides.

Heating the solutions causes the color to develop more rapidly, but is to be avoided. At 50° the same maximum is not reached. Sulfuric acid hastens the development of color, but fading occurs more quickly; nitric acid causes a yellow to green color; acetic acid retards or prevents appearance of the color. With high acidity there is a tendency to liberation of iodine.

The method will detect 0.5 ppm. The highest accuracy is obtained when 0.2 mg. of platinum is present in a 50-ml. sample. The optimum amount of hydrochloric acid for 0.05-0.2 mg. of platinum in 50 ml. is 0.5-1 ml. of 1:11 hydrochloric acid. In the absence of acid the full color does not develop for 16 hours. Fading, when it occurs, is not due to light.

Permanent standards made from cobalt sulfate and potassium dichromate are suitable. As cobalt standard, dissolve 10 grams of the sulfate hexahydrate and 1 ml. of concentrated sulfuric acid in water and dilute to 100 ml. This is equivalent to 5.9 grams of the anhydrous salt or 10.4 grams of the heptahydrate. As dichromate standard, dissolve 0.1 gram of potassium dichromate and 1 ml. of concentrated sulfuric acid in water and dilute to 100 ml. Add the dichromate standard to the cobalt standard until the desired tint is obtained. The 1-4 ppm. solutions are particularly easy to match. For 50 ml. samples the following mixtures are suitable.

Platinum in mg. per 50 ml.	*Cobalt Standard in ml. per 50 ml.*	*Bichromate Standard in ml. per 50 ml.*
0.1	3.5	1.5
0.2	9.0	3.0
0.3	14.5	4.5

Procedure. Transfer an aliquot of sample containing 0.01-0.1 mg. of platinum to a 25-ml. volumetric flask. Unless the acidity is accu-

[3] Lothar Wöhler, *Chem.-Ztg.* **31,** 938 (1907); B. G. Karpov and G. S. Savchenko, *Ann. secteur platine, Inst. chim. gén.* (U.S.S.R.) No. **15,** 125-8 (1938).

rately known, neutralize and add 0.5 ml. of 1:9 hydrochloric acid. Prepare a similar standard. Dilute each to about 22 ml. and add 0.5 ml. of a 5 per cent solution of potassium iodide. Dilute to volume, mix, and store in the dark.

Compare the colors after an hour, although it is then only about 90 per cent of the maximum. The rate of development after an hour's standing is very slow. The color does not develop as readily with fresh solutions as with those which are two weeks or more old, although the maximum is the same. An alternative to comparison with a standard is reading the transmittance with a blue-green filter.

Platinum by Reduction with Stannous Chloride

Platinum in solution may be determined by reducing a solution of platinic chloride to a yellow to orange color of chloroplatinous ion.[4] It is important that the acidity of sample and standard be the same. If the acidity is less than 0.1 N, precipitation may occur and the intensity of color may be low. A suitable final acidity is about 0.25 N, but the determination can be made at much higher concentrations. Doubling that acidity can be expected to reduce the color developed by less than 10 per cent. The color develops very quickly and is stable for at least a day.

Palladium gives a reaction of similar color intensity. By making the solution ammoniacal and then acidifying to about 1:12 with hydrochloric acid, that interference is avoided.[5] Ruthenium produces about 10 per cent of the color intensity developed by platinum, and rhodium and iridium give even lower levels of intensity. The reagent precipitates gold when present in significant amount. Copper alters the hue toward green but may be screened out.

A faint yellow color is shown by 0.01 mg. per ml. or 10 ppm. For more than 0.2 mg. of platinum micro gravimetric methods are more accurate. If the color is developed in 1:10 hydrochloric acid, or higher, it may be extracted with ethyl ether or ethyl acetate, detecting 0.03 mg. of platinum with an accuracy of 5 per cent.[6]

Procedure. *Direct Determination.* A fresh stannous chloride solution is required. For this dissolve 5 grams of crystallized stannous

[4] B. G. Karpov and G. S. Savchenko, *Ann. secteur platine, Inst. chim. gén.* (U.S.S.R.) No. 15, 125 8 (1938).

[5] H. Wölbling, *Ber.* 67B, 773 6 (1934).

[6] N. A. Figurovskiĭ, *Ann, secteur platine, Inst. chim. gén.* (U.S.S.R.) No. 15, 129 35 (1938).

chloride in 10 ml. of a stock hydrochloric acid solution containing 33 ml. of concentrated acid per liter. Transfer a sample containing 0.005-0.05 mg. of platinum to a 25-ml. volumetric flask. Unless the acidity is accurately known, neutralize and add 1 ml. of 1:1 hydrochloric acid. Prepare a similar standard. Dilute each to about 22 ml., and add 1 ml. of the fresh reagent. Dilute to volume and mix. Compare after 15 minutes by dilution with the stock hydrochloric acid solution or by balancing. After allowing that time for development, the color is constant for several hours. Excess of stannous chloride does no harm. Alternatively, read the transmittance with a blue filter.

Extraction. If the amount of platinum is below the lower limit specified, repeat, but this time add 5 ml. of 1:1 hydrochloric acid. When the color is fully developed, extract with 5 ml. of ethyl acetate and read the final color in that solvent.

MISCELLANEOUS

The reducing action of excess mercurous chloride on platinum, giving a precipitate colored by the reduced metal, is a somewhat inexact method using a series of standards.[7] For the test use the sample described under gold (page 511) and follow substantially the technic described in detail for palladium (page 533).

[7] Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* **6**, 437-9 (1934); *ibid.* **11**, 86-8 (1939).

CHAPTER 32

RHODIUM

RHODIUM is associated with platinum in many of its ores and alloys. It is lightly plated over metals for tarnish resistance, and therefore the plating baths require control analysis. The method of greatest importance is reduction with stannous chloride.

SAMPLES

Solids. Fuse a sample of suitable size with potassium acid sulfate. Extract the melt with water, filter, and dilute or evaporate the filtrate to a suitable volume for the use of aliquots.

Mixed Platinum Metals. The method of separation of rhodium from osmium, ruthenium, platinum, palladium, and iridium is given under platinum (page 513).

STANDARD

Dissolve 1.9229 grams of rhodium phosphate in water and dilute to 1 liter. Alternatively, fuse 10 mg. of rhodium metal powder with 2 grams of potassium bisulfate in a silica crucible until solution is complete. Take up in 1:30 sulfuric acid and dilute to 100 ml. Each ml. contains 1 mg. of rhodium.

RHODIUM BY STANNOUS CHLORIDE

The concentration of comparatively pure solutions of rhodium in hydrochloric acid is estimated by the rose-red color produced with stannous chloride[1] The first effect of adding the reagent to the boiling solution is production of a brown colloidal dispersion of the metal, analogous to the reactions of gold and platinum in the cold. The crimson color is probably due to the colloidal particles gradually going into solution in an acid medium. The greater the amount of rhodium present, the longer the time necessary for full development of the color.

[1] V. N. Ivanov, *J. Russ. Phys. Chem. Soc.* 49, 601 3 (1910); W. Singleton, *Ind. Chem.* 3, 121 (1927).

Dilution with water alters the color to yellow, but it can he diluted witb 1:5 hydrochloric acid witbout change of the dominant wave length. Either the red or the yellow color is suitable for colorimetric estimation,[2] hut the yellow is more sensitive photometrically. The metbod is similar to one in use for plotinum and several similor metals but is particularly sensitive when applied to rhodium.

Platinum may be prevented from interfering by odding sodium bisulfite so that a colorless complex ion forms.[3] Equally specific methods of avoiding interference by other metallic ions are not available ond so the rhodium must usually be isolated. As in tbe case of platinum tbe color may be extracted from 1:5 hydrochloric ocid solntion with solvents sucb as ethyl ocetate, giving a yellow color to the extract. Even without extraction tbe color is detectable below 1 ppm.

Procedure. Dilute or concentrate the sample to give 0.01-0.1 mg. of rhodium in a volume of 5 ml. If desirable, prepare o comparable standard. Add on equal volume of 20 per cent solution of stannous chloride in 1:3 hydrochloric acid. Fit the tuhe or flask with a long air condenser and heot in a boiling water bath for 1 hour. Before the end of that period the color should have reacbed a maximum intensity.

Yellow Color. Transfer the cooled solution to o 50-ml. volumetric flask and dilute to volume with water. Mix and let stand for 1 hour before comparing sample ond standard. Alternatively, read the transmittance with a blue filter.

Red Color. Transfer the cooled solution to a 50-ml. volumetric flask and dilute to volume with 1:5 hydrochloric acid. Compare the sample and standard or read the transmittance with a blue-green filter. No delay is necessary hefore reading. The red color is stable for honrs.

Rhomum as the Bromme

The addition of an excess of alkali metal bromide results in a colorless aqueous solution which when treated with dilute acid turns to a deep rose color suitahle for colorimetric estimation.[4] The method is accurate within 10 per cent.

[2] E. B. Sandell, "Colorimetric Determination of Traces of Metals," p. 385. Interscience Publishers, Inc., New York, N. Y. (1944).

[3] D. I. Ryabchikov, *J. Applied Chem.* (U.S.S.R.) 17, 284-6 (1944).

[4] Herbert E. Zschiegner, U. S. Patent 2,085,177 (1937).

Procedure. To 2-ml. aliquots of the sample and standard, add 2 ml. of 1:6 sulfuric acid and 2 grams of sodium bromide. Heat slowly and boil gently for 1 minute. Cool, dilute the standard to a known volume, and determine rhodium in the unknown by the dilution method or, alternatively, compare with permanent standards.

Miscellaneous

In comparatively pure solutions rhodium is estimated by the rose-red color in concentrated hydrochloric acid.[5] Many other metals interfere. In solutions containing 0.1 gram per liter in concentrated hydrochloric acid, platinum, nickel, gold and palladium give pale yellow, cobalt sky blue, copper, iron and osmium golden yellow, ruthenium and iridium brown.

[5] F. Mylius and A. Mazzucchelli, *Chem. News* 112, 90-1 (1915).

CHAPTER 33

IRIDIUM

Iridium is another metal associated with platinnm in many of its ores. When added to platinum it has a specific hardening function. Because of the comparative rarity of its occurrence, the methods of determination are strictly limited.

Samples

Platinum. Dissolve a 0.04397 gram sample of platinum containing iridium in 3 parts of concentrated hydrochloric acid and 1 part of concentrated nitric acid. Evaporate just to dryness. Add concentrated hydrochloric acid and again evaporate just to dryness. Take up with distilled water, neutralize with 1:1 ammonium hydroxide, and dilute to 100 ml. This is equivalent to a solution of 1 gram of ammonium chloroplatinate per liter if the platinum were pure.

Ammonium Chloroplatinate containing Iridium. Dissolve 0.1 gram in distilled water and dilute to 100 ml.

Solutions. If the solution contains mainly platinum and iridium, dilute or concentrate to approximately the concentration of the above solutions. If the iridium is not known to be in the tetravalent condition heat to and maintain at 70-80°. Pass chlorine gas through the solution until oxidation is complete. Remove excess chlorine by passing air or carhon dioxide through until all excess chlorine has been removed.

Mixed Platinum Metals. The method of separation of iridium from osmium, ruthenium, platinum, palladinm, and rhodium is given under platinum (page 513).

Standard

The determination of iridium is provided by a comparison against a platinum standard (page 519).

Iridium by Benzidine

Iridium gives a blue color with benzidine and acetic acid, similar to that given by platinum but deeper. This may be used to determine

iridium in platinum.[1] The iridium must be tetravalent. Oxidizing agents must be absent.

Procedure. Dissolve 1 gram of benzidine in 10 ml. of glacial acetic acid and 50 ml. of water. The solution should not be more than 24 hours old when used. To 10 ml. of sample solution containing about 1 mg. of platinum and iridium per ml. add 2 drops of the reagent. A blue color will develop at once. If the concentration of iridium is high, a blue precipitate will be formed. In that case dilute the sample to a standard concentration with 0.1 per cent pure iridium-free ammonium chloroplatinate solution. A suitable mixture when the iridium concentration in the sample is high is 90 ml. of standard platinum solution and 10 ml. of sample solution.

Compare the color of the treated sample with that of the standard platinum solution similarly treated by use of a balancing type of colorimeter. The amount of iridium present in the platinum-iridium solution is then obtained from Table 9.

TABLE 9. COLOR READINGS OF PLATINUM-IRIDIUM SOLUTIONS

Height of Platinum Standard in mm.	*Height of Sample Solution in mm.*	*Percentage of Iridium in Platinum*
100	95 ± 1	0.05
100	90 ± 1	0.1
100	81 ± 1	0.2
100	74 ± 1	0.3
100	69 ± 1	0.4
100	65.5 ± 1.5	0.5

MISCELLANEOUS

If a faintly acid solution of the colorless leuco compound of malachite green is treated with iridium in its higher stage of oxidation, the blue-green color is proportional to the iridium content.[2] Other oxidizing agents, such as gold in the auric form, must be absent. Interference by ferric iron is avoided by addition of fluoride. For the determination follow substantially the technic described for gold (page 512).

The red-brown color of chloriridic ion may be read. Adjust the acidity to correspond approximately to that of 1:10 hydrochloric acid and oxidize with chlorine as described under samples of solutions (page 526). Compare with a standard similarly treated. The method is not very sensitive.

[1] V. G. [illegible], Ann. inst. platine 4, 324-25 (1926).
[2] I. M. Kolthoff, [illegible] 5, 170 (1926).

CHAPTER 34

PALLADIUM

PALLADIUM is another of the platinum metals and generally has to be separated from them. It is also sometimes found as an alloying element with gold. Organic reagents such as *p*-nitrosodiphenylamine give colors suitable for its estimation.

SAMPLES

Metallic Platinum.[1] Dissolve 20 mg. of powdered metal in 6 ml. of concentrated hydrochloric acid and 2 ml. of concentrated nitric acid by heating. Add 10 mg. of sodium chloride and 3 ml. of concentrated hydrochloric acid, and evaporate to remove nitric acid. Repeat additions of hydrochloric acid and sodium chloride until nitric acid has been completely removed. Take up with a known amount of water.

Separation of Gold. To a 10-15 ml. aliquot of sample in hydrochloric acid solution, add sufficient 1:1 hydrochloric acid to bring the final acid concentration to 1:10. Transfer the solution to a small separatory funnel, add 10 ml. of ethyl acetate or ether, and shake for a few seconds. Draw off the acid layer and shake the solvent layer with 5 ml. of 1:10 hydrochloric acid. Combine the acid extracts. If necessary, carry out a second extraction with an additional 10 ml. of solvent and wash as before. Evaporate the aqueous solution to dryness on a steam bath and cool. Add 5 ml. of concentrated hydrochloric acid and evaporate so that only 1 drop remains. Dilute to a known volume and determine palladium.

Separation of Platinum.[2] Add 0.2 ml. of 0.7 per cent ferric nitrate to an aliquot of sample in hydrochloric acid containing 100 mg. of sodium chloride. Evaporate the solution to dryness, add 10 ml. of concentrated hydrochloric acid, and again evaporate to dryness. Add 4-5 drops of concentrated hydrochloric acid and 40 ml. of water, and heat the solution to boiling. Add 2 ml. of a filtered 10 per cent sodium bromate solution. Then add a 10 per cent solution of sodium bicarbonate

[1] John H. Yoe and Lyle G. Overholser, *J. Am. Chem. Soc.* 61, 2058-63 (1939).
[2] Raleigh Gilchrist and Edward Wichers, *ibid.* 57, 2565-73 (1935).

dropwise until a drop of the sample solution on a stirring rod turns red when tested with cresol red indicator. Add 2 ml. of the 10 per cent sodium bromate solution and boil for 15 minutes.

Filter through fritted glass and wash the precipitate with five 5-ml. portions of 1 per cent sodium chloride solution whose pH has been adjusted to 6-7. Dissolve the washed precipitate in 5 ml. of concentrated hydrochloric acid and wash the filter with water. Evaporate the solution to dryness on the steam bath, add 4-5 ml. of concentrated hydrochloric acid, and evaporate down to a drop. Cool and dilute to a known volume.

Mixed Platinum Metals. The method of separation of palladium from osmium, ruthenium, platinum, rhodium, and iridium is given under platinum (page 513).

Concentration by Sorption.[3] Use a sample containing 0.0001-0.01 mg. If oxidizing acids are present, add excess of 1:1 hydrochloric acid and evaporate to a small volume, repeating if necessary. Finally dilute to 25-75 ml. and have the solution 1:4-1:6 with hydrochloric acid. Add 1 ml. of 0.1 per cent sodium tellurite solution and, while agitating, 20 per cent stannous chloride in 1:6 hydrochloric acid until the tellurium appears as a precipitate. Add 5 ml. excess and heat to boiling until the precipitate is well coagulated.

Filter on an inorganic filter and wash with cold 1:10 hydrochloric acid, using a silica crucible as receiver. Dissolve the precipitate from the walls of the precipitation vessel and the filter with a warm mixture of 0.5 ml. of concentrated nitric acid and 1 ml. of concentrated hydrochloric acid. Wash the filter with hot water. Add 1 ml. of 0.4 per cent potassium sulfate solution and evaporate to dryness. Heat over a Meker burner for 10 minutes to volatilize all the tellurium. Add a drop of concentrated nitric acid and 2 drops of concentrated hydrochloric acid and again evaporate to dryness. Add about 1.5 ml. of warm 1:500 hydrochloric acid to take up the residue. After a few minutes, filter if necessary. Fuse 50 mg. of potassium pyrophosphate in the crucible until practically completely decomposed. To the cold melt add a drop of concentrated nitric acid and 2 drops of concentrated hydrochloric acid. Heat to dryness. Take up in 1 ml. of warm 1:500 hydrochloric acid, filter if necessary, and join with the previously described solution as sample.

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[3] E. B. Sandell, "Colorimetric Determination of Traces of Metals," p. 355. Interscience Publishers, New York, N. Y. (1944).

Standard

Dissolve 1 gram of metallic palladium in 12 ml. of concentrated hydrochloric acid and 4 ml. of concentrated nitric acid. Repeatedly add 10-ml. portions of concentrated nitric acid and evaporate to remove chlorine. Dilute to a liter, keeping the nitric acid concentration at approximately 1:10. This represeuts 1 mg. of palladium per ml.

Palladium by *p*-Nitrosodiphenylamine and Related Compounds

p-Nitrosodiphenylamine and related compounds such as *p*-nitrosoaniline, *p*-nitrosodimethylaniline and *p*-nitrosodiethylaniline react with palladous chloride in neutral or dilute acid solution to form colored complexes and may be used to determine small quantities of palladium.[4] *p*-Nitrosodiphenylamine forms a dark red solution, and in very high concentrations of the metal a purple-brown precipitate forms. A minimum of reagent in the presence of an acetate buffer prevents turbidity in the solution. Under these conditions, color develops more slowly.

p-Nitrosodimethylaniline and *p*-uitrosodiethylaniline, both of which form bright red complexes, have a faster reaction rate, greater stability, and a smaller temperature effect than *p*-nitrosodiphenylamine. They are also more applicable for spectrophotometric application. *p*-Nitrosoaniline, which is the least sensitive of the reagents mentioned, forms a dark brown complex. The complexes are of the $Pd(C_6H_5NHC_6H_4NO)_2Cl_2$ type. The *p*-nitrosodimethylaniline and *p*-nitrosodiethylaniline complexes are sparingly soluble in water, ethanol, and acetate buffers. The *p*-nitrosodiphenylamine complex however is insoluble in water and acetate buffers but readily soluble in ethanol. All the complexes are soluble in strong acids and bases. Absorption curves for the *p*-nitrosodimethylaniline and *p*-nitrosodiethylaniline complexes indicate that absorption due to the reagent may be practically eliminated at 525 mμ, but this is not true of *p*-nitrosodiphenylamine.

The presence of the acetate buffer salts in 0.05 *M* concentration, which is necessary to repress turbidity of *p*-nitrosodiphenylamine, or the presence of any small amount of salts, prevents the maximum color and sensitivity of the reaction from being obtained. Up to 0.006 per cent of sodium chloride solution is without effect on the *p*-nitrosodiphenylamine reagent, while up to 0.18 per cent of the salt solution may be present without effect in the presence of *p*-nitrosodimethylaniline and *p*-nitrosodiethylaniline. Furthermore, the latter reagents can tolerate a

[4] John H. Yoe and Lyle G. Overholser, *J. Am. Chem. Soc.* 61, 2058-63 (1939); *ibid.* 63, 3224-9 (1941).

slightly higher hydrochloric acid concentration. The intensity of color is also dependent on the pH of the solution as shown in Table 10.

TABLE 10. PROPERTIES OF PALLADIUM COMPLEXES

Reagent	*pH for Maximum Intensity of Reagent*	*pH for Maximum Sensitivity of the Complex*	*Optimum pH*	*Tolerance of N Hydrochloric Acid*
p-nitrosodiphenylamine	5-6	3.0 *turbid solution*	2.1	0.5 ml.
p-nitrosodimethylaniline *p*-nitrosodiethylaniline	0.9-5.2 (the most rapid change is from 0.9-3.0)	4.8 clear solution	4.8	0.75 ml.

Although the maximum intensity of the *p*-nitrosodiphenylamine reagent is at a pH of 3, solutions of the palladium complex above pH 2.1 become turbid. Therefore a pH of 2.1 is used, since this provides the maximum sensitivity obtainable in clear solution. The *p*-nitrosodimethylaniline and *p*-nitrosodiethylaniline reagents are only sparingly soluble in the buffer solution and probably form true solutions rather than colloidal suspensions. If the solution is strongly acid or alkaline, the colored complexes do not form at all.

As the temperature increases, the rate of development of the color of the *p*-nitrosodiphenylamine complex increases markedly, but above 50-60° the color fades completely. *p*-Nitrosodimethylaniline and *p*-nitrosodiethylaniline are less sensitive to temperature changes. The determination is usually carried out at room temperature. The reagent should be added to all solutions as nearly simultaneously as possible, and matching completed within an hour.

Using *p*-nitrosodiphenylamine reagent, estimation of 0.0005-0.050 mg. of palladium is possible and as little as 0.000005 mg. may be detected by the spot plate technique. Tests with the *p*-nitrosodimethylaniline and *p*-nitrosodiethylaniline reagents indicate that they conform to Beer's law up to 0.2 ppm.

Gold, iridium, and rhodium in concentrations of more than 1 ppm., and platinum in concentrations more than 20 ppm. introduce interference. Silver must be absent to prevent the formation of a turbid solution in the presence of chloride ion. Removal as the chloride results in occlusion of some palladium. If the silver concentration does not exceed 20 mg., it is advisable to determine palladium as the nitrate, using *p*-nitrosodiphenylamine reagent and a sodium acetate-nitric acid buffer for pH 1.2, or the *p*-nitrosodimethylaniline or *p*-nitrosodiethylaniline reagent

with a sodium acetate-nitric acid buffer for pH 4.8. The limiting concentrations for ions yielding colored solutions are as tabulated:

Ion	*p-Nitroso-diphenylamine (ppm.)*	*p-Nitroso-dimethylaniline (ppm.)*
Cobalt	10	10
Copper	50	3
Iron	30	2
Nickel	25	15

Even small quantities of ceric, bromate, iodide, cyanide, and other strong reducing and oxidizing ions must be absent.

Procedure. If *p*-nitrosodiphenylamine is to be used, prepare the reagent by dissolving 50 mg. in 500 ml. of 95 per cent ethanol and dilute to 1 liter. Prepare a buffer solution by adding 240 ml. of 1:11 hydrochloric acid to 200 ml. of 8 per cent anhydrous sodium acetate solution and dilute to 1 liter. The pH of this solution is 1.6, and when 25 ml. are diluted to 100 ml. the pH increases to 2.1.

If *p*-nitrosodimethylaniline and *p*-nitrosodiethylaniline are used, prepare the reagent by dissolving 25 mg. in 50 ml. of 95 per cent ethanol and dilute to 1 liter. Prepare a buffer solution for pH 4.8 by adding 80 ml. of 1:11 hydrochloric acid to 200 ml. of 8 per cent anhydrous sodium acetate solution and dilute to 1 liter.

Transfer an aliquot of sample containing 0.001-0.02 mg. of palladium to a 100-ml. volumetric flask. Pipet 25 ml. of the appropriate buffer solution into the sample and follow immediately with 2 ml. of reagent solution. Dilute the contents of the volumetric flask with water, mix thoroughly, and compare with standards or read the transmittance after allowing to stand at room temperature for 5 minutes for *p*-nitrosodimethylaniline and *p*-nitrosodiethylaniline reagents, and for 30 minutes for the *p*-nitrosodiphenylamine reagent. The former are stable for 3-4 hours, the latter for 1-2 hours. A 512-mμ filter is suitable for low concentrations, and a 585-mμ or 556-mμ filter for high concentrations. To correct for reagent absorption at low concentrations of palladium, a reagent solution may be used as standard.

Palladium by Mercurous Chloride

Mercurous chloride precipitates metallic palladium from a neutral or acid solution and, if no interfering substances are present, as little as

0.00005 mg. of the metal may be determined with an accuracy of ±3 per cent.[5] The colors produced by various concentrations of palladium are as tabulated.

Palladium (mg.)	*Color of Mercurous Chloride*
0.2	Very dark gray
0.05	Gray
0.01	Light gray
0.002	Cream-gray
0.0004	Grayish-cream
0.00005	Faint cream

Nitrates, persalts, iodides, free halides, stannous chloride, hypophosphites, arsenic, gold, selenium, tellurium, iodine, and large amounts of copper and iron interfere.

Procedure. If gold or platinum is present, separate as described under platinum (page 515). To a 25-ml. aliquot of solution, add 25 ml. of 10 per cent oxalic acid solution and boil. If any gold precipitates, filter. Add 2 ml. of 1:1 sulfuric acid to the filtrate to decompose oxalic acid and evaporate nearly to dryness. If any precipitation occurs during evaporation, dissolve in 3 ml. of 1:1 hydrochloric acid and add 50-75 mg. of potassium chlorate. Heat to drive off free chlorine and excess acid. Cool and dilute to about 40 ml. Add 10 ml. of a 25 per cent solution of copper sulfate pentahydrate. To 5 ml. of 1:20 hydrochloric acid add 0.1 gram of powdered mercurous chloride and 0.05-1 ml. of the test solution. Shake, allow to settle, and compare with similarly prepared standards.

Palladium by Nitric Acid

The yellow to brown color imparted by palladium to nitric and sulfuric acids in parting of precious metal beads is proportional to the amount of palladium present.[6] The method is usually applicable to solutions obtained in parting. They should not be exposed to light more than 2 days.

Ruthenium imparts a pink color in the presence of sulfuric acid. Excessive boiling with nitric acid dissolves sufficient platinum to make

[5] Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* 6, 437-9 (1934); *ibid.* 11, 86-8 (1939).

[6] F. C. Robinson, *Bull. Am. Inst. Mining Met.* 1926, No. 260.

the procedure unreliable. In nitric acid less than 10 times as much platinum as palladinm must be present, since platinum imparts a color of its own which will cause the result to he high. All other metals giving colors, such as copper, nickel and iron, must be removed during the assay and cupellation. Gold in the bead parted must be 10 times the amount of platinum and palladium together, and the silver 3 times the combined weight of these three. If not, add known amounts of gold and silver to the bead and recupel. The bead should be annealed before parting.

The method is ordinarily accurate to ±0.1 mg. of palladium for each determination. For more accurate results a special assay should be run and the bead parted in nitric acid only. That reduces the possible error to estimation in only the one solution.

Procedure. *Sulfuric Acid Parting Solution.* Part the metals as usual with sulfuric acid. Pour the solution into a white porcelain dish. If a yellow color is visible, it indicates the presence of more than the minimum detectable amount of palladium, 0.2 mg. Cool the acid solution and pour into a Nessler tube. Dilute to 50 ml. with concentrated sulfuric acid. In a similar tube place 45 ml. of concentrated sulfuric acid.

Prepare a special nitric acid as follows: Boil 2 liters of 1:1 nitric acid to remove nitrous fumes. When cool, add 0.8 ml. of concentrated hydrochloric acid. Mix well, add a solution of silver nitrate containing at least 1.2 grams of silver, and again mix well. Let stand overnight and filter. The filtrate should be clear and water white.

Prepare a standard solution of palladium in sulfuric acid as follows: Grind 0.1 gram of palladium sponge to a fine powder. Dissolve in the special nitric acid, adding about 0.5 gram of powdered silver to assist in solution. Add about 10 ml. of concentrated sulfuric acid and evaporate to sulfur trioxide fumes. When cool, dilute to 100 ml. with concentrated sulfuric acid. Each ml. of the resulting standard contains 1 mg. of palladium.

Add the standard solution of palladium in sulfuric acid until the color of the sample, as viewed lengthwise through the cyinder, is matched. Carefully dilute the duplicate with concentrated sulfuric acid, and more standard if necessary, to 50 ml., and compare horizontally as a check.

Nitric Acid Parting Solution. The procedure is the same when examining the nitric acid parting solution, except that special nitric acid and a solution of palladium in nitric acid are used in preparation of the duplicate.

Prepare a standard solution of palladium in nitric acid in a similar way to the sulfuric acid standard, except that evaporation to fumes is unnecessary and special nitric acid is used in place of sulfuric acid throughout. The nitric acid extracts of the fillet are joined as sample. The standard solutions are fairly constant if exposure to strong light is avoided. They should be checked from time to time by running determinations against known amounts of palladium.

If the amount of palladium present in either the sulfuric or nitric acid parting solution exceeds 5 mg., it must be diluted and the determination carried out on an aliquot.

MISCELLANEOUS

Potassium iodide imparts a deeper brown color to a platinum solution when palladium is present than when only platinum is present. The difference is sufficient to permit the estimation of palladium.[7] The amount of palladium in the platinum need not exceed 0.2 per cent. Iridium, rhodium and gold do not interfere.

[7] O. E. Zvyagintzev, *Ann. inst. platine* **1**, 361-6 (1926).

CHAPTER 35

OSMIUM

OSMIUM is another of the platinum metals, characterized by the volatility of its highest oxide. The lesser volatility of the lower oxides permits absorption in the presence of reducing agents. Its extremely poisonous nature is relatively unimportant because of the rarity of this metal. As might be expected with so rare an element, only a single method of determination is available.

SAMPLE

Meteoric Iron.[1] Heat a 1-gram sample with 10 ml. of 1:5 sulfuric acid at near the boiling point until no further reaction is visible. Decant this solution and reserve. Heat the remainder of the sample with 10 ml. of 1:1 hydrochloric acid until dissolved. Add 4 ml. of 1:1 sulfuric acid and evaporate to sulfur trioxide fumes. Chloride must be eliminated, as chloro-osmate is distilled only with great difficulty. Cool, add sufficient water to dissolve, and again evaporate to sulfur trioxide fumes. Take up the cooled mass in 10 ml. of water by warming and transfer to the distilling apparatus shown in Figure 21. Also transfer the original sulfuric acid solution to this apparatus. Add 5 per cent potassium permanganate solution dropwise, until a permanent pink is obtained. This oxidizes any remaining reducing substances. Add about 50 mg. of ferrous ammonium sulfate hexahydrate to destroy oxides of manganese and the excess permanganate. Unless they are removed, ruthenium will distill. Dilute to 35-40 ml., add a few beads or other

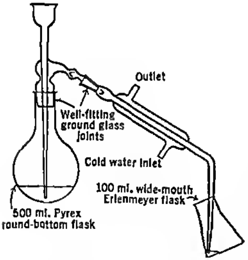

FIG. 21
Distilling Apparatus for Osmium

[1] E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.* 16, 342-3 (1944).

device to prevent bumping, connect with the condenser, and heat nearly to boiling to insure solution of oxides of manganese.

Put 10 ml. of 1:1 hydrochloric acid, freshly saturated with sulfur dioxide, in the receiver consisting of a cylinder calibrated at 20 ml. Add 15 ml. of concentrated nitric acid to the flask and distill so that 10 ml. of distillate is collected in 10-15 minutes. Use this as sample.

Mixed Platinum Metals. The method of separation of osmium from palladium, ruthenium, platinum, rhodium, and iridium is given under platinum (page 513).

Standard

As standard dissolve 1 gram of osmic oxide, OsO_4, in water and dilute to 1 liter. This contains 1 mg. per ml. If results are to be expressed as osmium, use 1.336 grams and the result will be 1 mg. of the element per ml.

Osmium by Thiourea

Osmium present as tetroxide in 1:2 hydrochloric acid gives a red color with thiourea, which is suitable for colorimetric estimation.[2] Isolation by distillation as the tetroxide is necessary and permits estimation of 0.001-0.002 mg. The presence of an interfering yellow color due to reaction of thiourea and sulfur dioxide often makes photometric reading definitely preferable to visual comparison. The system follows Beer's law.

Procedure. Transfer a sample or aliquot containing up to 0.05 mg. of osmium to a 25-ml. volumetric flask. This should be in 1:1 hydrochloric acid containing sulfur dioxide. Dilute to about 20 ml. with 1:1 hydrochloric acid and add 0.5 ml. of 10 per cent solution of thiourea. If chloro-osmate is present in the sample, add 0.1 ml. of 10 per cent stannous chloride solution in 1:5 hydrochloric acid. Heat in a boiling water bath for 10 minutes and cool. Otherwise skip the heating stage. Dilute to volume, mix, let stand for 5 minutes, and read with a green filter.

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[2] L. A. Chugaev, *Compt. rend.* 167, 235 (1918); *Z. anorg. allgem. Chem.* 148, 65 8 (1925).

CHAPTER 36

RHENIUM

RHENIUM is found most abundantly in molybdenite ores, its concentration ranging from 0.0001-0.01 per cent. This low concentration, combined with the fact that molybdenum undergoes reactions similar to those of rhenium, makes a thoroughly satisfactory determination difficult. The element was first discovered in 1925, and this probably accounts for the lack of sufficient justifiable work on the determination.

SAMPLES

Molybdenite Ores.[1] Treat 4 grams of finely powdered sample with 20 ml. of concentrated nitric acid. When frothing has ceased, add 5 ml. of fuming nitric acid. Stir occasionally until the reaction subsides. Heat on a hot plate at 80-90° until the red fumes have cleared.

If selenium is present, add two volumes of concentrated hydrochloric acid for each volume of the solution, thus making it 8-10 N with respect to hydrochloric acid. Add 1 gram of sodium sulfite and dissolve. Allow the solution to stand for 10 minutes. Filter and wash the residue well. If selenium is absent, add 50 ml. of concentrated hydrochloric acid. Evaporate slowly, adding further acid if necessary until no further chlorine is evolved. This may require 125-150 ml. of concentrated acid.

In either case, reduce the volume further to 25 ml. and cool. Add 75 ml. of concentrated sulfuric acid carefully to avoid excessive foaming due to escape of hydrogen chloride gas. Transfer the resulting solution, including any precipitated molybdic acid, to a distilling unit such as is shown in Figure 22.[2] Distill with a mixture of two parts of steam and one part of carbon dioxide or air, heating the solution to 260-270°, so that 250 ml. of distillate are obtained in 2.5 hours. Collect the distillate in a receiver immersed in an ice bath. The distillate contains sulfuric acid in a concentration low enough not to interfere, rhenium, and a few mg. of molybdenum.

Add dropwise a concentrated solution of bromine dissolved in 10 per cent aqueous sodium bromide solution to produce a faint yellow

[1] Clarence F. Hiskey and V. W. Meloche, *Ind. Eng. Chem., Anal. Ed.* 12, 503-6 (1940).

[2] Loren C. Hurd and Clarence F. Hiskey, *ibid.* 10, 623-6 (1938).

color in the distillate. This destroys any sulfur dioxide formed during the distillation process. Dilute to an appropriate volume and use an aliquot.

Pyrolusite.[3] To 100 grams of pulverized sample, add 50 ml. of water and 200 ml. of concentrated hydrochloric acid as rapidly as the frothing of the sample will permit. As soon as the reaction subsides,

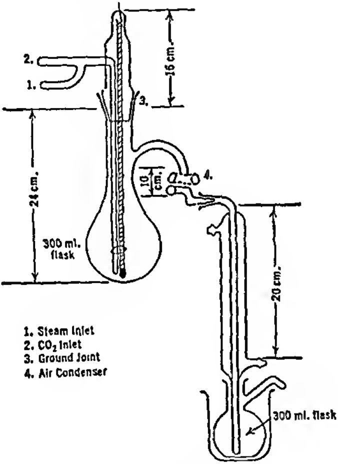

FIG. 22
Distilling Unit for Rhenium

place on a hot plate, and maintain at 60-80° until solution is complete, making additions of acid as necessary. Eight hours or more may be necessary. Cool, dilute to 300 ml., and filter through a Büchner funnel to remove insoluble silica. Return the precipitate and filter paper to the original flask. Add 10-15 ml. of water and 25 ml. of concentrated hydrochloric acid. Heat to boiling and filter. Combine the filtrates and add 20 per cent stannous chloride solution until the color changes from

[3] *Ibid.*

yellow-orange to the clear pink of manganese chloride, indicating that all the ferric ion has been reduced. Dilute to about 400 ml. and add 12 ml. of 20 per cent potassium thiocyanate solution. Add 10 ml. of 20 per cent stannous chloride solution. Shake in a separatory funnel and let stand for 5 minutes. Extract with ether until a colorless layer is obtained. Four 60-ml. portions of solvent generally suffice.

Transfer the combined extracts of rhenium and molybdenum oxythiocyanates to a distilling flask immersed in a 70° water bath, and distill until only 5-10 ml. of solution remains. To the concentrated extract add 15 ml. of 1:1 hydrochloric acid and remove the remaining ether by blowing a gentle current of air on the surface. Add 30 per cent hydrogen peroxide until all red, brown, or orange color has disappeared. Allow to stand for 10-15 minutes, adding hydrogen peroxide occasionally to prevent return of color. Dilute to 200 ml. with concentrated sulfuric acid and transfer to the distillation apparatus shown in Figure 22.

Heat the flask to 270-90° and pass in a mixture of steam and carbon dioxide for 2 hours until 250 ml. of distillate are collected in the ice-cooled receiver. Sulfur dioxide is usually present in the distillate. The presence of a large amount would indicate improper oxidation of the sample and would usually be accompanied by precipitation of sulfur in the distillate. Bubble bromine vapor through the distillate until it shows a faint yellow color. Dilute to a known volume and use as sample. It is free of manganese and molybdenum.

Standard

Dissolve 0.0155 gram of pure potassium perrhenate in water, add 5 ml. of 1:5 sulfuric acid, and dilute to 100 ml. This will contain 0.01 mg. of rhenium per ml. If a more dilute standard is required, dilute 10 ml. to 100 ml. with 1:100 sulfuric acid. This contains 0.001 mg. per ml.

Rhenium by Stannous Chloride and a Thiocyanate

When a perrhenate in hydrochloric acid solution is treated with stannous chloride and an alkali thiocyanate, the stannous chloride reduces the heptavalent ion to the hexavalent state,[4] and a stable yellow to yellow-brown color is produced which may be extracted with ether,

[4] Loren C. Hurd, *ibid.* 8, 11-15 (1936).

butyl acetate, or cyclohexanol for quantitative estimation of rhenium.[5] The compound formed is probably $ReO(CNS)_4$. The acidity, time, temperature, and concentration of reagents must be standardized. A similar reaction is obtained with molybdenum, necessitating its removal. Molybdenum forms a violet-red chloroform-soluble complex with ethyl xanthate while rhenium does not. Use has been made of this fact to separate the two elements, but it is not satisfactory for accurate quantitative work. A differential separation with mercury is acceptable.[6]

High concentrations of hydrochloric acid cause fading of the molybdenum thiocyanate complex, but not of the rhenium complex.[7] Interference of any molybdenum that may be carried over into the distillate in distillation methods for separation of rhenium may thus be eliminated by extending the time interval before colorimetric measurement is made. If very little rhenium is present, the thiocyanate complex may be concentrated by extraction with a few ml. of ether, and the extract treated with concentrated hydrochloric acid to bleach the molybdenum complex. This procedure will determine whether any color observed is due to rhenium or to traces of molybdenum thiocyanate. Although the method is of low accuracy, nevertheless it makes possible estimation of very small quantities of rhenium.

Platinum, rhodium, tungsten, copper, and large amounts of chromium and manganese also interfere. Oxidizing agents, excessive amounts of reducing agents, and high salt concentrations interfere. Reduction of iron by stannous chloride prevents its interference.

Measurements are made at 436 mμ. From 0.001-10 mg. of rhenium may be determined. The lower limit of sensitivity is 0.00005 mg. per ml.[8] When the reaction is applied to up to 0.5 mg. of rhenium, the optimum conditions are hydrochloric acid 2.0 per cent, potassium thiocyanate 0.4 per cent, and stannous chloride 0.2 per cent. Seven minutes should elapse before extraction, and the solution should be shaken no more than is necessary to produce uniformity.

Procedure. *By Extraction.*[9] Adjust the acidity of an aliquot of

[5] W. Geilmann, F. W. Wrigge and F. Weibke, *Z. anorg. allgem. Chem.* 208, 217-24 (1932); James I. Hoffmann and G. E. F. Lundell, *J. Research Natl. Bur. Standards* 23, 497-508 (1939).

[6] James I. Hoffman and G. E. F. Lundell, *J. Research Natl. Bur. Standards* 23, 497-508 (1939).

[7] C. F. Hiskey and V. W. Meloche, *Ind. Eng. Chem., Anal. Ed.* 12, 503-6 (1940).

[8] Loren C. Hurd and Bernard J. Babler, *ibid.* 8, 112-14 (1936).

[9] James I. Hoffman and G. E. F. Lundell, *J. Research Natl. Bur. Standards* 23, 497-508 (1939).

sample substantially free from molybdenum (page 485) so that the hydrochloric acid is present in a 1:5 ratio. Add 1 ml. of a 20 per cent solution of potassium thiocyanate and 1 ml. of stannous chloride solution prepared by dissolving 350 grams of the dihydrated salt in 200 ml. of 1:1 hydrochloric acid, cooling, and diluting to a liter with freshly boiled cold water. Shake slightly and allow to stand for 5 minutes. Add 20 ml. of ether, which has been previously shaken with 2 ml. of 20 per cent potassium thiocyanate solution, 2 ml. of the stannous chloride solution, 10 ml. of 1:1 hydrochloric acid, and 20 ml. of water to remove peroxides. Shake and separate the solvent layer. Drain the acid and, if more than 0.1 mg. of rhenium is present, repeat the ether extraction twice, using 15 ml. each time. Discard the acid solutions and combine the ether extracts.

Add 10 ml. of 1:4 hydrochloric acid and shake. Withdraw the acid layer and discard. Iron will have been so removed and thus possible later development of a pink color due to oxidation will be avoided. Dilute the ether layer to 50 ml. with the specially prepared ether and compare by balancing against a simultaneously prepared standard. Alternatively,[10] obtain the transmittance at 432 mμ. If molybdenum is present, let the developed solutions stand for one-half hour for that color to fade.

Rhenium by Sodium Tellurate and Stannous Chloride

The reaction between sodium tellurate and stannous chloride to form tellurium and stannic chloride is catalyzed by perrhenic acid and its salts.[11] A protective colloid such as gelatin or gum arabic will keep the tellurium in suspension, enabling the rhenium to be determined colorimetrically with a suitable light filter. The color is directly proportional to the time up to 2-4 hours so that solutions made up at different times may be compared. After 10-20 hours there is a drop in the rate of color formation. Nitric acid suppresses the reaction completely. Other acids change the color.

Maximum light absorption occurs at 430-470 mμ. No appreciable concentration of molybdenum is permissible. In the range cited in the procedure accuracy is to 10-20 per cent.

Procedure. Place aliquots of the sample solution, each to contain 0.0001-0.000001 mg. of rhenium, in three graduated test tubes. To one

[10] E. B. Sandell, "Colorimetric Determination of Traces of Metals," p. 379. Interscience Publishers, Inc., New York, N. Y. (1944).

[11] N. S. Poluektov, *J. Applied Chem.* (U.S.S.R.) 14, 695-702 (1941).

add a quantity of standard perrhenic acid solution equivalent to 0.00002-0.0001 mg. of rhenium, and to the second a quantity equivalent to 0.000002-0.00001 mg. of rhenium. Dilnte each to 1.5 ml.

Prepare a reagent hy mixing 5 ml. of 0.5 per cent sodium tellurate solution, 2 ml. of 45 per cent tartaric acid solution, 1.5 ml. of 0.5 per cent gelatin solution, and 1.5 ml. of a stannous chloride solution containing 100 grams of granulated tin in 250 ml. of concentrated hydrochloric acid. Add 1 ml. of this reagent, mix, and allow to stand for 2 bours. At the same time set aside a hlank of 1.5 ml. of water and 1 ml. of reagent. Examine the solutions at 430-470 mμ or, if no eolor develops, allow to stand until the next day and eompare. Determination is preferably made photometrically, hut may be obtained by the dilution method, using as the diluent 10 ml. of reagent diluted to 25 ml. By the former method rhenium is ealeulated at follows:

$$x = aEx/(E_{x+a} - E_x)$$

where

a = amount of standard rhenium solution added to the sample
E_x = extinction of the test solution
E_{x+a} = extinction of the test solution with added standard rhenium solution.

Without Extraction.[12] Dilute the sample solution containing 0.01-0.1 mg. of rhenium to ahout 50 ml. and add successively 20 ml. of concentrated hydrochloric acid, 2 ml. of 20 per cent sodium thiocyanate solution, and 2 ml. of 20 per cent stannous chloride solution. Mix well and let stand for an hour for the color due to molybdenum to fade. Read the color at 430-470 mμ, or compare with a standard simultaneously prepared.

Miscellaneous

The pink or red complex formed with α-benzildioxime and stannous chloride in 1:3-1:1 sulfuric acid[13] is soluble in amyl and isoamyl alcohols and acetates. To 1 ml. of sample containing sulfuric acid to make it 1:2 with respect to that acid, add 2 mg. of α-benzildioxime, 0.5 ml. of isoamyl alcohol, and 0.2 ml. of 15 per cent stannous chloride in 1:3 sulfuric acid. Heat nearly to boiling for 3 minutes and add 1 ml. of water. Compare the color with standards. More than 10 times as much molybdenum will interfere.

[12] Clarence F. Hiskey and V. W. Meloche, *Ind. Eng. Chem., Anal. Ed.* 12, 503-6 (1940).

[13] Suzanne Tribalat, *Compt. rend.* 224, 469-71 (1947).

CHAPTER 37

POTASSIUM

Potassium occurs in a large total amount, over 2 per cent, in the earth's crust and is widely distributed. Typical samples vary from minerals, glass, and water, to soil and soil extracts as well as biological and food samples. Plants, for example, contain around 5 times as much potassium as sodium, thereby accentuating the importance in soils. The classical method of determination is by the color as the chloroplatinate, which may be intensified 100-fold by reduction or conversion to the iodoplatinate. The most widely used method is an indirect one depending on preparation of potassium sodium cobaltinitrite and determination of either cobalt or nitrite by conventional methods. Because of possible inaccuracies arising from variable composition of the cobaltinitrite precipitate, it is open to more question than the other methods. There are various other methods, as would be expected from the importance of potassium.

Samples

Silicates.[1] To 1 gram of 100-mesh sample in a platinum crucible, add 0.5-1.0 ml. of 72 per cent perchloric acid. Place the crucible on a hot plate and heat until the last trace of perchloric acid has been evaporated. This destroys all organic matter. Cool and add 8 grams of a 2:1 mixture of sodium carbonate and lithium carbonate. Mix thoroughly and cover with an additional gram of the carbonate mixture. Cover the crucible and fuse in a muffle furnace at 500-600° until all bubbling has ceased. Remove the crucible and rotate to distribute the fused mass on the sides of the crucible as the melt cools. Place the crucible in a beaker, add 75 ml. of 1:2 hydrochloric acid, and cover. To hasten solution, break any silica shell that forms and, if solution is very slow, place on a steam bath. When the carbonates have dissolved, wash the crucible and lid with water and remove. Evaporate the solution to dryness on a steam bath. Cool, treat with 10 ml. of 72 per cent perchloric acid, and evaporate to dryness on a hot plate. Cool and add 1 ml. of concentrated hydrochloric acid, 0.5 ml. of concentrated nitric

[1] J. E. Gieseking and H. J. Snider, *Ind. Eng. Chem., Anal. Ed.* 9, 232 (1937).

acid, and an additional 10 ml. of 72 per cent perchloric acid. Again evaporate to dryness. This destroys any ammonium salts as well as dehydrating the silica. Treat the residue with 25-50 ml. of 1:20 hydrochloric acid and heat on the steam bath to dissolve the crust of iron salts. Filter, decanting from residual silica, wash the silica residue twice with 1:5 hydrochloric acid, and continue to wash with hot water until the washings come through chloride-free. Set this solution aside.

Dry the paper, remove the dried silica, and ignite the filter paper in platinum. Add the silica to the residue in the platinum crucible. Treat with 2 ml. of water, 3 drops of 72 per cent perchloric acid, and 5 ml. of 48 per cent hydrofluoric acid. Evaporate to dryness, cool, and repeat the treatment without the addition of water. Dissolve the residue in 2 ml. of hot 1:20 hydrochloric acid. Combine with the filtrate from the silica. Evaporate until salts begin to crystallize, transfer to a 100-ml. volumetric flask, and dilute to volume. Use 10-ml. portions as aliquots, preferably making the determination by the cobaltinitrite method, after neutralizing with sodium hydroxide and acidifying with acetic acid.

Ash from Organic Materials.[2] Ash a sample which will contain 0.04-1.0 mg. of potassium. If the content is larger, it can be corrected by aliquoting later. Dissolve the soluble portion in 5 ml. of 1:99 hydrochloric acid. Add a drop of phenolphthalein indicator solution and make definitely alkaline with 10 per cent sodium hydroxide solution. Heat to boiling for 15 minutes to drive off ammonia. Interfering ferric and vanadium ions will also be precipitated along with the insoluble matter already present. Filter and wash well with about 20 ml. of hot water. Make the filtrate acid to litmus paper with acetic acid and either use as sample or dilute to a known volume for the use of aliquots.

Soils.[3] *Aqueous Extract.* Extract 30 grams of dry soil containing not more than 6 per cent of calcium oxide,[4] with 100 ml. of water for 0.5 hour. Filter and add 2 ml. of concentrated hydrochloric acid to an aliquot of the solution containing 1-10 mg. of potassium oxide. Evaporate to dryness and ignite at a dull red heat to destroy organic matter and volatilize ammonium salts. Take up the residue with 25 ml. of 1:3 hydrochloric acid. Heat on the steam bath and filter out the insoluble residue. Wash the residue on the filter and dilute the filtrate and

[2] C. P. Sideris, *ibid.* **9**, 145-7 (1937); *ibid.* **14**, 821-2 (1942).
[3] Paul L. Gow, *Hawaiian Planters' Record* **35**, 401-9 (1931).
[4] Th. Arnd and E. Leisen, *Bodenkunde u. Pflanzenernähr.* **30**, 51-62 (1942).

washings to a suitable volume for use of aliquots. Use the chloroplatinate method.[5] This sample is also suitable for estimation of nitrate by reduction to ammonia and for ammonia present as such.

Ammonium Acetate Extract.[6] Remove ammonia from 100 ml. of an extract made with 7.6 per cent amomnium acetate at pH 6.8 by evaporation to dryness, neutralize with 4 per cent sodium hydroxide solution, and add 25 ml. of 1:99 acetic acid. Precipitate potassium with sodium cobaltinitrite in this medium. Approximately the same results are obtained by extraction of soil with 0.05 *N* hydrochloric acid or with 0.2 *N* barium chloride solution adjusted to pH 8.1 by addition of triethanolamine.

For proper determination of potassium it is essential that the soil give an extract that is clear and colorless.[7] The use of animal charcoal to decolorize is not permissible as it sorbs ingredients to be determined. On moor soils the limits have been set at not over 6 per cent of calcium oxide in the dry soil and an extract color not deeper than that of 5 ml. of 1:3 hydrochloric acid and 10 grams of ferric chloride hexahydrate in 500 ml. of water.

Solutions Containing Phosphates or Iron. Prepare magnesia mixture by dissolving 55 grams of crystallized magnesium chloride and 70 grams of ammonium chloride in 650 ml. of water. Dilute to 1 liter with 1:3 ammonium hydroxide. Render 10 ml. of sample solution alkaline with 1:3 ammonium hydroxide. Add 1 ml. of magnesia mixture and let stand in the cold for 24 hours. This precipitates phosphates and iron but no potassium. Heating, or too much magnesia mixture, will precipitate potassium. Centrifuge and remove the liquid. Stir the precipitate with 3 ml. of 1:3 ammonium hydroxide, centrifuge, and decant. Wash once more.

Evaporate the solution and washings to dryness, and heat until no more fumes are evolved. The ammonia and ammonium salts are volatilized. Dissolve the residue in 4 ml. of water and transfer to a tube for precipitation as cobaltinitrite.

Water.[8] If the potassium concentration exceeds 2 ppm., the sample

[5] F. Malychin, *Chem. Listy* 36, 2-6 (1942).

[6] N. J. Volk and E. Truog, *J. Am. Soc. Agron.* 26, 537-46 (1934); cf. Hans Riehm, *Z. Pflanzenernähr., Düngung Bodenk.* 39, 309-14 (1935).

[7] F. Malychin, *Chem. Listy* 36, 2-6 (1942).

[8] Rex J. Robinson and Garth L. Putnam, *Ind. Eng. Chem., Anal. Ed.* 8, 211-13 (1936).

may be used without concentrating, applying the silver cobaltinitrite procedure. If it contains less than 2 ppm., add 10 ml. of 5 per cent acetic acid to each 100 ml., and concentrate by heating. In dilution of standards if the water is soft, use distilled water. For hard water, prepare a synthetic standard of the same hardness. For this, use a stock containing calcium 195 ppm., magnesium 53.3 ppm., sodium 780 ppm., ferric ion 35 ppm., silicate 150 ppm., chloride 1080 ppm., sulfate 582 ppm., and an acidity of 0.8 per cent acetic acid.

For water containing up to 1 ppm. of potassium use a series-of-standards method with 0.5 gram of sodium chloride added per sample of 5 ml.[9]

Biological Samples in General. Care is necessary in the preparation of sample to insure that potassium is not lost by volatilization in ashing or by mechanical loss in transfer. Ashing in nickel [10] or quartz [11] centrifuge tubes at 465° in a muffle furnace in the presence of red mercuric oxide is satisfactory. Methods of precipitation of protein include the addition of thorium nitrate,[12] trichloroacetic acid,[13] or zinc sulfate.[14]

Wet ashing [15] with perchloric acid and nitric acid, as in the preparation of plant materials which follows (page 550) is permissible. When the reaction is complete, volatilize the excess acid as there described. Take up the cooled residue in water and add methyl red indicator. Add a saturated solution of sodium carbonate until neutralized. If a precipitate is formed, centrifuge, decant the upper layer, and set aside. Dissolve the precipitate in 1:4 acetic acid and precipitate again. Centrifuge and combine the decantates as sample.

Blood.[16] Pipet 0.4 ml. of sample into a nickel or quartz ashing tube. Dilute to 2 ml. and add 2 ml. of a 3 per cent potassium-free gelatin solution and 2 ml. of 95 per cent ethanol. Evaporate to dryness in an electric oven at 85°. Moisten the residue on the walls of the tube with 4-5 drops of absolute ethanol. Over this sprinkle from a spatula about 2-2.5 grams of red mercuric oxide. Place horizontally in a muffle and ash 10-12 hours at 465°. Cool, moisten the ash with ethanol, and add a

[9] M. Kriventzov, *Pedology* (U.S.S.R.) 1947, 52 4.
[10] Precision Scientific Company, 1750 North Springfield Avenue, Chicago, Ill.
[11] Macalaster Bicknell Company, New Haven, Conn.
[12] M. B. Strauss, *J. Biol. Chem.* 118, 331 (1937).
[13] A. Heiduschka and H. Ober, *Biochem. Z.* 292, 191-5 (1937).
[14] L. Jendrassik and F. Takács, *ibid.* 274, 194-9 (1934).
[15] Rubens Salomé Pereira, *J. Biol. Chem.* 160, 617 29 (1945).
[16] Peter Waldemar Salit, *ibid.* 136, 191 200 (1940).

small amount of mercuric oxide as before. Ash for another 10-12 hours. Cool and pipet 8 ml. of cold water into the tube. Stir for a few minutes with a glass rod, rubbing the sides frequently. Stopper and centrifuge for 10 minutes at 4000 rpm. or for 15 minutes at 2000 rpm. Decant the supernatant liquid, and aliquot 5 ml. of it into a 15-ml. centrifuge tube. This represents 0.25 ml. of whole blood. Evaporate the contents of the centrifuge tube in an electric drying oven and use the residue as the sample, preferably carrying out the determination by the chloroplatinic acid method.

Alternatively,[17] transfer 7-8 ml. of sample to a 50-ml. volumetric flask containing 25 ml. of water. Determine the exact amount of blood by difference in weight. Add 1-2 drops of amyl alcohol, then slowly 12 ml. of a 12 per cent trichloroacetic acid solution. Allow to stand for 10 minutes, dilute to volume, and centrifuge. Evaporate a 1-ml. aliquot in a platinum crucible, add 3 drops of 72 per cent perchloric acid, and ash carefully at not over a dull red heat. Cool, add 1 drop of 72 per cent perchloric acid and 1 drop of water, and heat to dryness. Use the residue as sample, determining potassium by the chloroplatinic acid method.

Results by the sodium cobaltinitrite method are the same on trichloroacetic acid filtrates as on ashed plasma.[18] Analogously trichloroacetic acid filtrates from serum, plasma, or whole blood[19] are suitable for use by the silver cobaltinitrite method.

Animal Serum.[20] Transfer 2 ml. of sample to a nickel or quartz centrifuge ashing tube. Add 3 ml. of 95 per cent ethanol and evaporate to dryness in an electric oven at 85°. Ash as described in the procedure for blood (page 547) beginning with "Moisten the residue on the walls of the tube . . ." After "Ash for another 10-12 hours," cool, and dissolve in 6 ml. of water. Stir and centrifuge for 10 minutes at 4000 rpm. or for 15 minutes at 2000 rpm. Pour the supernatant liquid into small centrifuge tubes and use a 5-ml. aliquot, representing 1.6667 ml. of original serum. Evaporate the contents of the centrifuge tube in an electric drying oven and use the residue as aliquot, carrying out the determination by the chloroplatinic acid method.

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[17] A. Heiduschka and H. Ober, *Biochem. Z.* 292, 191-5 (1937).

[18] A. D. Marenzi and R. Gerschman, *Anales farm. bioquim.* (Buenos Aires) 3, 107-11 (1932).

[19] John E. Harris, *J. Biol. Chem.* 136, 619-27 (1940).

[20] Peter Waldemar Salit, *ibid.* 136, 191-200 (1940).

Human Serum.[21] Pipet a 1-1.5-ml. sample into a nickel or quartz centrifuge ashing tube. Add 5 ml. of 85 per cent ethanol and evaporate. Ash as described in the procedure for blood (page 547) beginning with "Moisten the residue on the walls of the tube . . ." After "Ash for another 10-12 hours," dissolve in 6 ml. of cold water, centrifuge for 10 minutes at 4000 rpm., or for 15 minutes at 2000 rpm. and pour the supernatant liquid into small centrifuge tubes. Use an aliquot of 5 ml., equivalent to 0.8333 ml. if the original sample was 1 ml., and 1.25 ml. if the original sample was 1.5 ml. Evaporate the contents of the centrifuge tube in an electric drying oven and use the residue as sample, carrying out the determination by the chloroplatinic acid method.

Plasma.[22] Plasma should be separated immediately after blood is taken, since potassium diffuses from the cells to the plasma on standing. To 0.3 ml. of sample in a 15-ml. conical quartz centrifuge tube, add 0.3 ml. of 1:5 sulfuric acid which contains 42.65 grams of anhydrous sodium sulfate per liter, and 0.1 ml. of concentrated nitric acid. The sodium sulfate gives a larger, more flocculent precipitate, and limits creeping of the potassium salt. Place the tube in a boiling water bath for 30-60 minutes. Transfer to a sand bath at 120° until only a dark brown drop of solution remains in the bottom of the tube. Take the temperature by inserting the thermometer in the sand to the same depth as the tube. Remove the tube, add 2 drops of concentrated nitric acid, and replace in the sand bath for evolution of nitrogen oxides. Then push the tube deeper into the sand to a point where the temperature of the tube tip is at least 250°. If the solution is not colorless after an hour's digestion, add another drop of concentrated nitric acid. Digest for 2-4 hours or overnight. Place in a muffle furnace and heat gradually to 400-500°. Maintain at this temperature for 5 hours. Use the white ammonia-free ash thus obtained as the sample, dissolving it in the same quartz centrifuge tube. Determine the potassium by the chloroplatinate method.

Urine.[23] Dilute a 5-ml. sample to 100 ml. Pipet 5 ml. of the diluted solution, representing 0.25 ml. of original sample, into a nickel or quartz centrifuge ashing tube. Add 2 ml. of 3 per cent potassium-free gelatin solution. Evaporate to dryness in an electric oven at 85° and ash the residue as described under whole blood (page 547) beginning with "Moisten the residue on the walls of the tube . . ." After "Ash for

[21] Peter Waldemar Salit, *ibid.* 136, 191-200 (1940).
[22] Robert Mayo Tenery and Carl E. Anderson, *ibid.* 135, 659-69 (1940).
[23] Peter Waldemar Salit, *ibid.* 136, 191-200 (1940).

another 10-12 hours," cool, and pipet 10 ml. of water into the tube to dissolve the ash. Stir for a few minutes with a stirring rod, rubbing the sides frequently. Stopper and centrifuge for 10 minutes at 4000 rpm. or 15 minutes at 2000 rpm. Pour off the supernatant liquid and pipet a 5-ml. portion of it into a 15-ml. centrifuge tube. This represents 0.125 ml. of original sample. Evaporate the contents of the centrifuge tube in an electric oven and use the residue as sample. Make the determination by the chloroplatinic acid method.

Feces.[24] Transfer 5 grams of sample to a 100-ml. lipless glass cylinder. Dilute to 100 ml. and close with a rubber stopper. Shake well and allow to stand overnight at room temperature. Shake again and transfer a 5-ml. aliquot, equivalent to 0.25 gram of original sample, to a nickel or quartz centrifuge ashing tube, using a calibrated glass tube for transfer. Add 2 ml. of a 3-per cent potassium-free gelatin solution and evaporate to dryness in an electric oven at 85°. Ash the residue as described for blood (page 547) beginning with "Moisten the residue on the walls of the tube . . ." After "Ash for another 10-12 hours," cool and pipet 10 ml. of cold water into the tube. Stir for a few minutes, rubbing the sides frequently. Stopper and centrifuge for 10 minutes at 4000 rpm., or 15 minutes at 2000 rpm. Decant the supernatant liquid, and aliquot 5 ml., equivalent to 0.125 gram of original sample, to a 15-ml. centrifuge tube. Evaporate the contents of the centrifuge tube in an electric drying oven and use the residue as sample, carrying out the determination by the chloroplatinic acid method.

Milk.[25] To 5 ml. of sample add dropwise 0.5 ml. of 3 per cent acetic acid and 5 ml. of 96 per cent ethanol. Dilute volumetrically to 25 ml. Allow to stand for 1 hour and filter through a dry paper to remove milk protein. Use aliquots of the filtrate to determine potassium, preferably by the sodium cobaltinitrite method.

Plant Materials.[26] To a 4-gram sample of material, add 10 ml. of concentrated nitric acid. Cover with a watch glass and heat gently on a hot plate until rapid initial reaction subsides. Bring to a boil and evaporate to 0.5 ml. Cool and add 10 ml. each of 1:1 nitric acid and

[24] *Ibid.*

[25] Masayoshi Sato and Kiichi Murata, *J. Agr. Chem. Soc. Japan* 13, 318-22 (1937).

[26] G. Frederick Smith, "Mixed Perchloric, Sulfuric, and Phosphoric Acids and Their Applications in Analysis," p. 13. G. Frederick Smith Chemical Co., Columbus, Ohio (1935).

72 per cent perchloric acid. Cover with a watch glass and boil gently until the solution is colorless, or almost so, to remove organic matter. Condensing fumes should wash down and destroy any organic matter on the sides of the beaker. Cool and wash down the sides of the beaker to dissolve adhering salts. Maintain just below boiling to evaporate to dryness and ignite at a dull red heat to remove ammonium salts. Cool, add 5 ml. of 1:1 hydrochloric acid and 10 ml. of water, and heat to dissolve the salts. Filter into a volumetric flask. Wash the residue thoroughly with hot water and combine the washings with the filtrate. Dilute to volume for use of aliquots. The solution so obtained is suitable for estimation of calcium, magnesium, and phosphorus, as well as potassium.

Alternatively,[27] to 0.2 gram of dried tissue, add 3 ml. of a solution containing 32 grams of salicylic acid per liter of concentrated sulfuric acid, mix, and allow to stand for 30 minutes. Add 4 drops of a 50 per cent sodium thiosulfate monohydrate solution and 5 ml. of a solution containing 2.4 grams of selenium oxychloride per liter of concentrated sulfuric acid. Agitate, heat slowly to boiling on a hot plate in a hood, and digest until clear. Cool and wash the neck of the flask with a small stream of water. If the solution is not colorless, add 2 drops of 10 per cent perchloric acid solution and mix. Heat gently, but do not boil, until the solution is colorless. Cool and dilute to 100 ml. Evaporate a 25-ml. aliquot of this solution to dryness on a hot plate and heat in a muffle furnace at low red heat to remove ammonium salts and excess sulfuric acid. Cool and take up with 2 drops of concentrated hydrochloric acid and 10 ml. of water. Transfer quantitatively to a 25-ml. volumetric flask and dilute to volume for use of aliquots for determination of potassium by the sodium cobaltinitrite method.

As another technic,[28] transfer 0.1 gram of dried ground sample or the equivalent of fresh material to a flask. Add 2 ml. of concentrated sulfuric acid, swirl, and allow to stand for 10 minutes. Heat for 3 minutes over a blue 1.25-cm. Bunsen flame. A short funnel may be used as a reflux. Dense fumes will be evolved. Raise the flame to 2.5 cm. in height and continue heating for 2 minutes. Cool and add 0.5 ml. of 30 per cent hydrogen peroxide solution. Place over a very low flame until bubbling begins. Remove until bubbling subsides, then return to the flame. When the hydrogen peroxide no longer causes bubbling, raise the flame to bring the mixture to a boil.

[27] Omer J. Kelley, Albert S. Hunter, and Athan J. Sterges, *Ind. Eng. Chem., Anal. Ed.* 18, 319-22 (1946).

[28] Robert H. Cotton, *ibid.* 17, 734-8 (1945).

The solution first becomes lighter and then darker. Cool to room temperature and add 0.25 ml. of 30 per cent hydrogen peroxide solution. Repeat the procedure previously outlined starting at "Place over a very low flame . . ." If after the boiling period the solution is yellow, add 3 drops of 30 per cent hydrogen peroxide; if darker, add 5 drops. Repeat the heating as before. If, on cooling, the solution is not nearly colorless, repeat. When colorless or almost so, boil for 3 minutes to expel all the peroxide. Cool, add 10 ml. of water, and transfer to a 100-ml. volumetric flask. Dilute to volume for the use of aliquots. Use aliquots of this solution for potassium, phosphorus, and nitrogen.

Transfer the aliquot, usually 10 ml., to a 30-ml. Vycor crucible. Evaporate on a steam bath to about 0.5 ml. Transfer to a hot plate or asbestos pad and heat slowly to produce fuming but no spattering. When fuming has almost ceased, rotate over a free flame to drive off sulfuric acid and ammonium sulfate adhering to the sides of the vessel. Avoid heating higher than to a dull red heat. Cool the dry residue, which need not be white, and wash down the sides of the crucible with 3 ml. of hot water to give the sample for use. This is suitable for evaporation to dryness and development of the color with dipicrylamine.

The sulfur as sulfate, calcium, magnesium, sodium, and potassium, from plant tissue are isolated as solution C in the determination of lead (page 30). Take an aliquot.

Fruit and Fruit Products.[29] Dry ash, take up the ash in concentrated hydrochloric acid, and evaporate to dryness. Add 5 ml. of 1:9 hydrochloric acid to the residue and filter into a volumetric flask of a size corresponding to the potassium and phosphorus pentoxide content. Wash the dish and filter until the sample is made up to volume. Ignore any flocculent precipitate which separates on washing. Remove an aliquot for determination of phosphorus and filter the remaining solution for determination of potassium by the cobaltinitrite method.

Standard

Potassium Chloride. Dissolve 1.907 grams of potassium chloride in water and dilute to 1 liter. Each ml. contains 1 mg. of potassium. Dilute 100 ml. of this to 1 liter to give a standard containing 0.1 mg. of potassium per ml. or 10 ml. to 1 liter to give 0.01 mg. of potassium per ml.

Potassium Sulfate. Dissolve 0.2228 gram of potassium sulfate in

[29] Harold W. Gerritz, *J. Assoc. Official Agr. Chem.* **25**, 443-7 (1942).

water and dilute to 1 liter. This contains 1 mg. of potassium per ml. For lesser concentrations dilute as described for the chloride.

Potassium by Chloroplatinic Acid

The yellow-orange color of potassium chloroplatinate may be read as such,[30] the yellow color on reduction with stannous chloride read,[31] or for maximum sensitivity the reading is of the reddish orange color of potassium iodoplatinate developed from the chloroplatinate in potassium iodide solution.[32] Unlike sodium cobaltinitrite and silver cobaltinitrite which form complexes of varying composition, depending on temperature and relative concentrations of sample and reagent, the potassium chloroplatinate has a definite composition.[33] Several improvements in the methods of preparation of samples permit the determination of 0.03-0.30 mg. of potassium, especially in biological samples.[34]

Ammonium ions, which form a similar insoluble chloroplatinate, iron, copper, and ferrocyanide ions, and ethanol interfere.[35] Ethanol and ammonia are destroyed, and copper and iron form insoluble oxides in the ashing procedure generally used for the preparation of sample.

Measurement of the yellow-orange color formed by the chloroplatinate complex itself provides a convenient method for the determination of potassium, in all but very minute quantities.[36] Although almost a hundred times less sensitive, the method has greater chemical stability and a faster rate of color development than the iodoplatinate reaction. A precision of 2 per cent can be obtained with samples containing 0.2 mg. of potassium or more. Slight deviations from Beer's law occur, probably because of hydrolysis of the complex ion. If the concentration range is up to 80 ppm. per liter, determinations with a 30-mμ slit are made, preferably at 410 mμ; up to 250 ppm. per liter, the preferred wave length is 470 mμ. The color appears immediately and is stable for 2 months. Reduction of the chloroplatinate with stannous chloride is applicable in the range of 1-10 ppm. of potassium.

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[30] M. F. Adams and J. L. St. John, *Ind. Eng. Chem., Anal. Ed.* 17, 435-6 (1945).

[31] E. Biilmann, *Z. anal. Chem.* 39, 284 (1900); L. A. Hill, *J. Am. Chem. Soc.* 25, 990-2 (1903); A. Nemec, *Biochem. Z.* 189, 50-6 (1927).

[32] Alfred T. Shohl and Helen B. Bennett, *J. Biol. Chem.* 78, 643-51 (1928).

[33] Peter Waldemar Salit, *ibid.* 136, 191-200 (1940).

[34] W. V. Consolazio and J. H. Talbott, *ibid.* 126, 55-61 (1938); Robert Mayo Tenery and Carl E. Anderson, *ibid.* 135, 659-69 (1940).

[35] F. K. Cameron and G. H. Failyer, *J. Am. Chem. Soc.* 25, 1063 (1903).

[36] M. F. Adams and J. L. St. John, *Ind. Eng. Chem., Anal. Ed.* 17, 435-6 (1945).

The greatest sensitivity is when the iodoplatinate is at 490 mμ.[37] Use of the colored solution of reagent rather than the solvent in the reference cells extends the concentration range and increases the sensitivity. Standard solutions may be read with an error of 1 per cent. In low concentrations of potassium, preparatory ashing leads to errors of 12 ppm. These errors may be caused by volatilization, solution of potassium in the glass apparatus, impure water, differences in solubility due to variations in room temperature, and loss of iodine from the final solution. However, if run against standards, determinations may be made with an accuracy of ±3 per cent. Within the given range the reaction follows Beer's law.

Procedure. Transfer the aliquot of sample solution containing 0.1-10 mg. of potassium to a centrifuge tube and adjust the acidity to that of 1:10 hydrochloric acid. If the sample is a residue, dissolve it in the minimum amount of 1:10 hydrochloric acid. Usually the sample solution will be not over 1 ml. For each ml. of sample solution add 0.5 ml. of 20 per cent chloroplatinic acid solution in 1:11 hydrochloric acid. Mix and allow the tube to cool in a refrigerator for 5 minutes. Pipet in 5 times the volume of refrigerated absolute ethanol,[38] saturated with chloroplatinic acid, and stir vigorously. Rinse the stirring rod with a few ml. of absolute ethanol. Stopper the tube with a cork and allow to stand in ice water in a refrigerator for 20 minutes. At the same time refrigerate the cups of the centrifuge. Insert the tubes and centrifuge for 10 minutes at 4000 rpm. or 15 minutes at 2000 rpm. Pour off the supernatant layer and invert the tubes over a piece of filter paper for 5-10 seconds. Dry the mouth of the tube with filter paper and add 5 ml. of refrigerated 95 per cent ethanol. Stir with a glass rod, wash the rod with 1 ml. of ethanol, and place in refrigerated centrifuge cups as before. Centrifuge, pour off the supernatant layer, and invert the tube over filter paper for 5-10 seconds. Dry the mouth of the tube with filter paper and dry horizontally in an electric oven for 10 minutes at 65°. This serves to remove all the ethanol which might interfere, as by later reduction of the iodo compound. Dissolve the contents of the tube in 5 ml. of water, stirring constantly. Transfer to a 100-ml. volumetric flask. Wash the tube into the flask several times.

Direct Reading. If the potassium is high enough, dilute to volume and read at 410 mμ or 470 mμ according to concentration. The sensitiv-

[37] *Ibid.*

[38] W. V. Consolazio and J. H. Talbott, *J. Biol. Chem.* 126, 55-61 (1938).

ity will be increased about 100-fold by proceeding to development of the iodoplatinate or by reduction with stannous chloride.

Development as Iodoplatinate. Dilute the combined solution and washings to about 90 ml., mix, and add 4 ml. of 72 per cent potassium iodide solution. Dilute to volume, stopper, and shake well. Allow to stand in a dark place at 68° for 10 minutes. Cool to room temperature by placing in a cold water bath for 1 hour. The addition of 4-ml. of 1:10 hydrochloric acid may replace the heating process to hasten the color development. Heat causes a more rapid release of iodine, and the color must therefore be read in a short time. Compare colorimetrically with standards simultaneously prepared by mixing portions of standard potassium chloride solution and reagent, or read photometrically around 490 mμ.

Reduction by Stannous Chloride. Dilute the solution of chloroplatinate to 80 ml. Add 10 ml. of 1:5 hydrochloric acid and 5 ml. of 10 per cent stannous chloride solution in 1:5 hydrochloric acid. Dilute to volume and read the transmittance with a blue filter.

Potassium by Sodium Cobaltinitrite

The most widely used method of determination of potassium is by precipitation as the potassium-sodium cobaltinitrite [39] and the subsequent determination of the amount of the precipitate. This is by estimation of the cobalt content [40] according to conventional methods; or of the nitrite content, often by formation of an azo dyestuff; or of the reducing value for an oxidizing agent.[41] These methods are gravimetric or volumetric for substantial amounts of potassium but colorimetric for small amounts. Colorimetric determination of the nitrite is very sensitive but is often subject to instability of the azo dye.

An aqueous reagent made by forming sodium cobaltinitrite is used because maximum precipitation is obtained in a medium that is barely acid with acetic acid.[42] The determination is influenced by the sodium concentration of the medium,[43] changes in the pH value, variations in

[39] R. H Adie and T. B. Wood, *J. Chem. Soc.* **77**, 1076-80 (1900); Benjamin Kramer and Frederick F. Tisdall, *J. Biol. Chem.* **46**, 339-49 (1921); N. J. Volk, *J. Am. Soc Agron.* **33**, 684-9 (1941).

[40] C. P. Sideris, *Ind Eng. Chem., Anal. Ed.* **9**, 145-7 (1937).

[41] I. W. Wander, *ibid.* **14**, 471-2 (1942).

[42] J. E. Schueler and R. P. Thomas, *ibid.* **5**, 163 5 (1933).

[43] Rex J Robinson and James Hauschildt, *ibid.* **12**, 676-7 (1940).

the state of hydration of the precipitate, rate of addition of ethanol and mixing, temperature changes, ammonia present, and the composition of the complex, which may be represented by the general formula $(KNa)_3Co(NO_2)_6$. The precipitate may range in composition from monopotassium disodium cobaltinitrite, $KNa_2Co(NO_2)_6$, to dipotassium monosodium cobaltinitrite $K_2NaCo(NO_2)_6$, and is usually a mixture of the two, the ratio varying somewhat with the conditions of precipitation. At 6°, in carefully regulated ethanol solution, a precipitate is obtained that corresponds to the formula, $K_{1.57}Na_{1.43}Co(NO_2)_6$.[44] These latter conditions tend to yield more precipitate and to form larger, more easily filtered crystals with a higher sodium content. At room temperature, the compound precipitated has been calculated to be most closely represented by the formula, $K_{1.35}Na_{1.65}Co(NO_2)_6$. By running a standard concurrently and by carefully controlling conditions, comparable results are obtained.

To avoid introduction of additional sodium ions, a zinc cobaltinitrite precipitate may be used.[45] An alternative is the use of silver cobaltinitrite which is presented as a separate method.

Modifications increase the sensitivity of the sodium cobaltinitrite reagent, which is cheaper than the chloroplatinic acid reagent.[46]

From 5-20 ppm. of potassium are determined, within 1 mg. For smaller quantities, a standard amount of potassium should be added to the aliquot, and later deducted from the results. Addition of sodium acetate prevents the precipitation of protein and excess cobaltinitrite by adjusting the pH on the alkaline side of the isoelectric point of the sample.[47] The acetate ion also serves to prevent the precipitate from adhering to the walls of the vessel.[48]

Gum arabic solution aids in the formation of a more uniform precipitate.[49] If determinations are conducted in a room where the temperature is subject to great variations, separate standard curves should be drawn for every 5°. Sensitivity is increased by precipitating in 30 per cent by volume of ethanol.[50] If the determination is made at 4-6°, as little as 0.003 mg. of potassium can then be determined, under con-

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[44] J. E. Scheuler and R. P. Thomas, *ibid.* 5, 163-5 (1933).

[45] Jane Adams, Martha Hall and W. F. Bailey, *ibid.* 7, 310-11 (1935).

[46] Robert H. Cotton, *ibid.* 17, 734-8 (1945).

[47] William S. Hoffman, *J. Biol. Chem.* 120, 57-61 (1937).

[48] Rex J. Robinson and James D. Hauschildt, *Ind. Eng. Chem., Anal. Ed.* 12, 676-7 (1940).

[49] Benjamin Wolf, *ibid.* 16, 121-3 (1944).

[50] F. H. L. Taylor, *J. Biol. Chem.* 87, 27-32 (1930).

ditions where no precipitate forms at room temperature. Isopropyl alcohol has also been used as a medium.

To overcome difficulties with fine precipitates which are troublesome to filter, and which vary in composition, a solution of sodium cobaltinitrite may be used as a precipitating reagent in the presence of nitric acid.[51] Subsequent oxidation of the precipitate with a potassium dichromate solution makes possible colorimetric determination of potassium. The yellow to yellow-green color is quite stable for several days if protected from dust and strong light. Application has been made to soil extracts[52] and plant tissue.[53] This is shown as a separate variation of the procedure.

Ammonium,[54] iron, aluminum, manganese, barium, calcium, magnesium, and copper interfere.[55] Formaldehyde prevents interference from ammonia,[56] but this reduces the sensitivity for the determination of minute quantities of potassium. Ammonia is readily removed by evaporating with dilute sodium hydroxide solution.

Turbidimetrically, the presence of suspended material acts as a nucleus for the formation of cobaltinitrite crystals and yields nonreproducible results.[57] Turbidimetric determinations may be made by adding formaldehyde to a dilute acetic acid solution of sample, followed by a sodium cobaltinitrite-sodium acetate reagent, and reading the turbidity of the resulting complex suspended in absolute ethanol.[58] The formaldehyde eliminates interference of 50 ppm. of ammonia, and up to 4000 ppm. of calcium. Temperature control within 0.5° is essential. Large concentrations of ammonium ion increase the intensity, but sodium hypochlorite corrects this.[59] More than 60 ppm. of sulfate ion decreases the intensity.

Another turbidimetric method involves shaking the sample with an acetic acid solution of sodium nitrate, and determining the turbidity produced when a sodium cobaltinitrite ethanol reagent is added.[60]

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[51] L. V. Wilcox, *Ind. Eng. Chem., Anal. Ed.* **9**, 137-8 (1937).

[52] I. W. Wander, *ibid.* **14**, 471-2 (1942).

[53] R. Q. Parks, S. L. Hood, Charles Hurwitz and G. H. Ellis, *ibid.* **15**, 527-33 (1943).

[54] R. F. Reitemeier, *ibid.* **15**, 393-402 (1943).

[55] L. T. Bowser, *U. S. Dept. Agric. Bur. Chem., Bull.* **152**, 45-50 (1912).

[56] Benjamin Wolf, *Ind. Eng. Chem., Anal. Ed.* **15**, 248-51 (1943).

[57] Rex J. Robinson and James D. Hauschildt, *ibid.* **12**, 676-7 (1940).

[58] G. M. Volk, *Soil Sci. Soc. Florida, Proc.* **3**, 99-101 (1941); Michael Peech and Leah English, *Soil Sci.* **57**, 167-95 (1944).

[59] J. Tinsley and N. H. Pizer, *J. Soc. Chem. Ind.* **64**, 182-7 (1945).

[60] K. Krumins, *J. Landw. Riga* No. **7**, (1933).

Potassium has been estimated in the cobaltinitrite precipitate by almost every method used for cobalt. Direct colorimetric[61] and photometric[62] estimation of cobalt at 610 mμ is possible by dissolving the precipitate in 27 per cent hydrochloric acid. Accuracy in the determination of the green to blue-green cobalt chloride color ranges from 1-2 per cent for 0.025-0.5 mg. Measurement of the green color of cobalt acid carbonate detects 4 ppm. of cobalt without interference from chloride.

In acetate buffered solutions, the disodium salt of 1-nitroso-2-hydroxy-3,6-naphthalene-disulfonic acid, Nitroso R salt, reacts with the cobalt in the cobaltinitrite precipitate to give a stable intense wine-red coloration.[63] Accurate results are obtained if the quantity of cobalt lies between 0.025 and 0.25 mg., or equivalently for 0.008-2.0 mg. of potassium. A photoelectric colorimeter permits this range to be extended to 0.0005-0.015 mg. of potassium.[64]

The addition of dimethylglyoxime and sodium sulfide results in a brown color suitable for colorimetric purposes. Conversion of the cobaltinitrite precipitate to colloidal cobalt sulfite by means of alkali sulfite is another means of determining potassium colorimetrically.[65] Treatment of the cobaltinitrite precipitate, freed from excess reagent, with ammonium sulfide permits comparison with similarly prepared standards.[66] The cobaltinitrite precipitate when treated with indole in acid solution forms the nitrosoindole for the estimation of 0.001 mg. of potassium per ml. with an accuracy of 2 per cent.[67] The red color of the indole complex is rather fugitive, and the extinction curve follows Beer's law only at 0.001-0.03 mg. of potassium per ml. The ammonium salt of 2-hydroxynaphthalene-1-azonaphthalene-4-sulfonic acid is also

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[61] A. D. Marenzi and R. Gerschman, *Anales farm. bioquím.* (Buenos Aires) 3, 107-11 (1932).

[62] A. D. Marenzi and R. Gerschman, *ibid.* 9, 85-90 (1938).

[63] C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* 9, 145-7 (1937); A. D. Marenzi and C. E. Cardini, *Anales farm. bioquím.* (Buenos Aires) 12, 32-9 (1941); J. Fielding Reed, A. Mehlich, and J. R. Pilard, *Soil Sci. Am., Proc.* 9, 56-60 (1944).

[64] C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* 14, 821-2 (1942).

[65] A. M. Alekseeva, *Bull. biol. med. exptl. U.R.S.S.* 1, 301-2 (1936).

[66] V. D. Marza and L. Chiosa, *Compt. rend. soc. biol.* 117, 524-6 (1934); V. D. Marza and A. V. Blinov, *Bull. soc. Roumaine Neurol., Psychiatrie, Psychologie Endocrinol.* 15, 221-32 (1934); V. D. Marza, *Bull. histol. appl. physiol. path. tech. microscop.* 12, 58-72 (1935); 13, 62-71 (1936).

[67] Margarete v. Wrangell (Fürstin Andronikov) and H. Beutelspacher, *Z. anal. Chem.* 90, 401-17 (1932).

used, preferably in dilute acetic acid solution to minimize interference from iron.[68]

Procedure. Prepare a solution of the reagent as follows: Dissolve 23 grams of sodium nitrite in 50 ml. of water and add 16 ml. of 1:2 acetic acid. Dissolve 3.5 grams of cobalt nitrate in this solution and allow to stand for a day. Filter and dilute the filtrate to 100 ml. Transfer an aliquot containing 0.02-1.0 mg. of potassium to a centrifuge tube and dilute or evaporate to 1 ml. Similarly treat an appropriate standard if the color is not to be read photometrically.

If ammonium ion is present, dilute the sample to 5 ml., and add 0.2 ml. of a 4 per cent sodium hydroxide solution. Boil until just dry, add 5 ml. of water, and boil again until almost dry. Dissolve in not more than 0.5 ml. of water. Add 1 drop of 0.01 per cent methyl orange solution in water, and neutralize with 1:17 acetic acid. Pipet 2 ml. of reagent into the centrifuge tube and swirl to mix. Place in a refrigerator at 5° for 3 hours, swirling several times during this period.

Wash the upper walls of the tube with 0.5 ml. of water but do not mix with the precipitation liquid. Centrifuge at 3000 rpm. for 10 minutes and drain for 5 minutes. Wipe the mouth of the tube, stir the precipitate, and wash the tube walls with two 3-ml. portions of a 70 per cent ethanol solution. Centrifuge and drain for 5 minutes after each washing. The final washings should not be yellow. This gives the precipitate to be determined by one of the methods which follow.

Development as Cobalt Chloride.[69] Dry the precipitates from the sample and a standard at 100° for 2-3 hours. Cool and dissolve in 3 ml. of concentrated hydrochloric acid by warming gently. Cool, transfer to comparison tubes, and compare by dilution with absolute ethanol to prevent hydrolysis, or by balancing. The standards may be kept about 1 week. Amounts of 0.025-0.5 mg. of potassium are estimated with an error of about 4 per cent.

Development as Basic Carbonate.[70] Dissolve the precipitates from sample and standard in 2-ml. portions of 1:1 hydrochloric acid. Evaporate to dryness and dissolve in 2 ml. of water. Transfer to 10-ml. volu-

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[68] Josef Tischer, *Mikrochemie, Festschr. von Hans Molisch* 1936, 418 35.

[69] F. Lebermann, *Biochem. Z.* 150, 548-59 (1924); Maurice Delaville, *Compt. rend. soc. biol.* 101, 1082 3 (1929).

[70] A. Blanchetière and J. M. Pirlot, *Compt. rend. soc biol.* 101, 858 60 (1929); Anthony A. Albanese and Dorothy L. Wagner, *J. Lab. Clin. Med.* 30, 280-4 (1945).

metric flasks and add 0.5 ml. of 3 per cent hydrogen peroxide. Dilute nearly to 10 ml. with a saturated solution of potassium or ammonium bicarbonate. Mix well and dilute to volume with water or bicarbonate solution. Mix and compare.

Development with Nitroso R Salt. This is the disodium salt of 1-ni--troso-2-hydroxy-3,6-naphthalene disulfonic acid.[71] To the cobaltinitrite precipitate in the centrifuge tube, add 5 ml. of 1:17 sulfuric acid. If the precipitate is very large, add 10 ml. of acid. Place on a steam bath for 5-10 minutes to dissolve the precipitate. Transfer quantitatively to a 100-ml. flask, and dilute to volume. Transfer an aliquot containing 0.025-0.100 mg. of cobalt to a graduated tube. Usually a 20-ml. aliquot will be satisfactory. Add 2 ml. of a sodium acetate solution prepared by dissolving 54.4 grams of the trihydrated salt in water, heating on a steam bath, diluting to 100 ml., and filtering. Dissolve 1 gram of nitroso R salt in 70 ml. of water and add 30 ml. of iron-free acetone. Add 1 ml. of this reagent to the sample solution, shake for 5 seconds, and compare the red color colorimetrically against a standard cobalt chloride solution similarly developed. Alternatively read photometrically with a 470-mμ filter. If the ratio of 1:17 sulfuric acid to the sodium acetate buffer is more than 1:2, the solution will be too acid and the color will not develop. A few drops of 40 per cent sodium hydroxide will correct this.

Development with Cysteine Hydrochloride and Hydrogen Peroxide. Cysteine combines with cobalt to give a blue-green cobaltobiscysteinate.[72] Dissolve the precipitates from sample and standard in 1-ml. portions of 1:2 hydrochloric acid and evaporate to dryness. Dissolve the residues in 5 ml. of 12.5 per cent potassium pyrophosphate solution. Add 5 ml. of a 0.35 per cent solution of cysteine hydrochloride. Add 2.5 ml. of 0.1 per cent hydrogen peroxide solution. Dilute to 25 ml. or other suitable volume and compare.

Development with Choline Hydrochloride and Potassium Ferrocyanide. Colorimetric[73] or photometric[74] determination of the emerald

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[71] A. D. Marenzi and C. E. Cardini, *Anales farm. bioquim.* (Buenos Aires) 12, 32-9 (1941); C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* 9, 145-7 (1937); *ibid.* 14, 821-2 (1942).

[72] Maxwell P. Schubert, *J. Am. Chem. Soc.* 53, 3851-61 (1931); Albert E. Sobel and Benjamin Kramer, *J. Biol. Chem.* 100, 581-71 (1933).

[73] H. R. D. Jacobs and William S. Hoffmann, *J. Biol. Chem.* 93, 685-91 (1931).

[74] A. Eden, *Analyst* 68, 167-71 (1943).

green color formed on the addition of 1 per cent choline hydrochloride and 2 per cent sodium ferrocyanide solutions yields good results for 0.05-0.25 mg. of potassium. Photoelectric measurements require the use of a blue filter [75] and are accurate to 3 per cent.

Dissolve 5 grams of choline hydrochloride in a minimum of absolute ethanol, filter, and add excess ether to precipitate. Filter through a suction funnel, wash with ether, and dry in a desiccator. Dissolve 0.2 gram in 50 ml. of water. Recrystallize potassium ferrocyanide from a boiling, saturated, aqueous solution by cooling. Collect on a suction funnel and dry. Dissolve 0.4 gram in 50 ml. of water.

Pipet 5 ml. of water vigorously into the precipitate so as to stir it up. Place the tube in a boiling water bath to dissolve the precipitate and cool. Pipet 2 ml. of each of the reagent solutions into the sample solution, dilute to 12 ml., and mix by inversion. Centrifuge if turbid. Read with a 580-mμ filter after 15-30 minutes and compare with a calibration curve. The color developed conforms to Beer's law.

Development with Thiocyanate in Acetone.[76] Formation of the stable blue potassium cobaltithiocyanate results on the addition of ammonium thiocyanate and acetone. A yellow light filter is employed if the color is very deep. Up to 5 mg. of potassium may be determined with a maximum error of 3.8 per cent.

Dissolve the precipitates of sample and standard in 0.5-ml. portions of 1:4 sulfuric acid by warming on the water bath. Transfer with 75 per cent acetone solution to 25-ml. volumetric flasks. Add 5 ml. of a 5 per cent solution of ammonium thiocyanate in acetone, dilute to volume with 75 per cent acetone solution, and compare.

Development with Sulfanilic Acid and α-Naphthylamine.[77] Dissolve a cobaltinitrite precipitate containing not more than 0.22 mg. of sodium nitrite, in 0.4 per cent sodium hydroxide solution. Add the solution to 1 ml. of 1 per cent sulfanilic acid in 30 per cent acetic acid and 1 ml. of 0.3 per cent α-naphthylamine solution. After 10 minutes, compare the red color with similarly prepared standards or read photometrically using a 530-mμ filter.

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[75] A. H. Sanford, C. Sheard and A. E. Osterberg, *Am. J. Clin. Path.* 3, 405 (1933); William S. Hoffman, *J. Biol. Chem.* 120, 57-61 (1937); R. F. Reitemeier, *Ind. Eng. Chem., Anal. Ed.* 15, 393-402 (1943).

[76] F. Alfred Uhl, *Z. anal. Chem.* 123, 322-33 (1942).

[77] K. A. J. Wretlind, *Acta. Physiol. Scand.* 1, 43-8 (1940).

Procaine may replace the sulfanilic acid.[78] Another alternative reagent [79] is sulfanilamide and *N*-(1-naphthyl)ethylene diamine. It is sensitive to the nitrite combined with 0.002 mg. of potassium in 100 ml. of solution. This is probably the most satisfactory method based on nitrite determination.

Development with Salicylic Acid and α-Naphthylamine.[80] Mix a solution of 2.5 grams of sulfanilic acid in 750 ml. of 10 per cent acetic acid with a solution prepared by boiling 1 gram of α-naphthylamine with 100 ml. of water and adding 750 ml. of 10 per cent acetic acid. This reagent should be colorless and will keep well in a dark bottle in a cool place.

Add 5 ml. of the reagent to the cobaltinitrite precipitates from sample and standard. Heat for 2 minutes in boiling water. Transfer the resulting red solutions to 100-ml. flasks. Add 5 ml. of the reagent to the residue and repeat. Continue so long as any residue remains. Cool, dilute to the mark with cold reagent, and compare.

A similar reagent [81] consists of 5 grams of sodium naphthionate and 2.5 grams of β-naphthol in 500 ml. of water. After shaking and filtering, the reagent is unchanged if not exposed to light. In the light it develops a rose color. This detects 0.001-1.0 mg. of potassium.

Development with Dimethylglyoxime and Sodium Sulfide.[82] Add 2 ml. of 5:2 nitric acid to the precipitates from sample and standard and warm until dissolved. To 1.5 grams of sodium acetate in each of two 50-ml. volumetric flasks, add the solutions of potassium cobaltinitrite and wash water. To each flask add 20 ml. of water, 5 ml. of a 1 per cent alcoholic solution of dimethylglyoxime, and 2 ml. of a 1 per cent sodium sulfide solution. After 3 minutes heat both in boiling water for 15 minutes, cool, and dilute to 50 ml. Compare in a colorimeter.

Development with Phenoldisulfonic Acid.[83] Wash the cobaltinitrite

[78] L. Jendrassik and F. Takács, *Biochem. Z.* 274, 194-9 (1934).

[79] Joseph M. Looney and Cora G. Dyer, *J. Lab. Clin. Med.* 28, 355-63 (1942).

[80] Miklos Dreguss, *Biochem. Z.* 233, 375-80 (1931).

[81] Joseph Tischer, *Biochem. Z.* 238, 148-61 (1931); *Mikrochemie, Festschr. von Hans Molisch* 1936, 418 35; F. Alten and H. Weiland, *Z. Pflanzenernähr., Düngung Bodenk.* 34A, 108-10 (1934).

[82] Masayoshi Sato and Kiichi Murata, *J. Agr. Chem. Soc. Japan* 13, 318-22 (1937).

[83] E. M. Emmert, *Proc. Am. Soc. Hort. Sci.* 44, 381-3 (1944); *ibid.* 45, 311-12 (1945).

precipitate with 70 per cent ethanol. Boil the precipitate with 5 ml. of a 4 per cent sodium hydroxide solution and filter. Alternatively, add 2 ml. of 40 per cent sodium hydroxide solution, then 0.5 ml. of 1:1 sulfuric acid. When all the precipitate has darkened, dilute to 5 ml. and filter. Cool and to a 0.5-ml. aliquot, add 0.05 mg. of sodium perchlorate and 1 ml. of fuming sulfuric acid as rapidly as possible without causing excessive boiling. To the clear solution add 1 ml. of phenoldisulfonic acid (page 792) and 15 ml. of water. Add 50 per cent sodium hydroxide solution until a permanent yellow color develops and compare with a series of standards similarly treated. The yellow color is proportional to the amount of soluble nitrogen present. The error is about 2 per cent.

Development by Stannous Chloride Reduction. Dissolve the residue from the tube with 5 ml. of hot 1:1 hydrochloric acid, running the solution into a 25-ml. volumetric flask. Wash well with hot water and cool. To this solution add 1 ml. of a solution of 75 grams of stannous chloride in 40 ml. of concentrated hydrochloric acid. A yellow to brown color will develop. After 15 minutes dilute to volume and compare with a standard developed at the same time.

Oxidation Procedure. Transfer a 10-ml. aliquot of almost neutral sample, containing 1-7 mg. of potassium, and a potassium reference standard to 2 test tubes. Add 1 ml. of 1:15 nitric acid, mix, and add 5 ml. of freshly filtered 20 per cent sodium cobaltinitrite solution (page 559). Mix and let stand at 20° for 2 hours. Wash the walls of the sample and standard tubes with 0.01 *N* nitric acid. Centrifuge at 2200 rpm. for 10 minutes, pour off the supernatant liquid, and drain. Wash the precipitate with 15 ml. of 0.01 *N* nitric acid, mix the precipitate thoroughly with wash solution, centrifuge, and drain.

Add 10 ml. of potassium dichromate solution, containing 4.9035 grams of salt per liter of solution, to the cobaltinitrite precipitate Add 5 ml. of concentrated sulfuric acid and mix well. The heat of reaction should give a clear solution. Cool to room temperature in a water bath and transfer the solution quantitatively to a 100-ml. volumetric flask. Dilute to volume and read colorimetrically using a 425-mμ filter.

Potassium by Silver Cobaltinitrite

Determination of very small quantities of potassium is made more

feasible by the silver cobaltinitrite reagent.[84] The composition of the precipitate approximates most closely the formula $K_{1.35}Ag_{1.65}Co(NO_2)_6$, which checks the composition found for the corresponding sodium compound (page 556). Inconstant composition of the precipitate, loss of precipitate during washing, and excess silver ions lead to inaccurate results. The excess of silver ions should only be slight, the silver nitrate concentration not more than 0.05 per cent.

While practically any reagent for development of color from the sodium potassium cobaltinitrite precipitate should be applicable, a mixture of α-naphthylamine and sulfanilic acid has proved a very sensitive means of determining the nitrite content of the precipitate. The mixture is stable for a 2-week period if stored in the absence of light. If the additions are made separately, the color is more intense, within certain limits, the longer the interval between the addition of the sulfanilic acid and α-naphthylamine. The use of a 10 per cent acetic acid solution prevents the formation of a reddish brown precipitate in the presence of large quantities of nitrite. Beer's law is valid for 0.00002-0.0002 mg. of potassium per liter. Sulfanilamide and *N*-(1-naphthyl)ethylenediamine determine the nitrite concentration with a sensitivity of 0.02 ppm. of potassium.[85] Beer's law is followed up to 0.01 mg. of potassium in the final solution. The color is also developed with thiocyanate (page 561). The complex may be decomposed and the mixed sulfides of cobalt and silver used for estimation.[86]

The silver cobaltinitrite method is adaptable to photoelectric colorimetry by the use of a band between 580 and 656 mμ. A combination of Corning glasses 245, 965 and 978 is suitable. The calibration curve approaches a straight line for 0.05-0.5 mg. of potassium.[87] The curve shifts as the reagent ages, indicating the desirability of the use of fresh reagent. As little as 0.02 mg. per ml. of potassium can be determined with an accuracy of 3-5 per cent. Acetone may be used as a wash solution to overcome any tendency of the silver potassium cobaltinitrite

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[84] F. Breh and Oliver H. Gaebler, *J. Biol. Chem.* 87, 81-9 (1930); Rex J. Robinson and Garth L. Putnam, *Ind. Eng. Chem., Anal. Ed.* 8, 211-13 (1936). A. M. Ismail and H. F. Harwood, *Analyst* 62, 443-52 (1937); R. Truszkowski and R. L. Zwemer, *Biochem. J.* 30, 1345-53 (1936); 31, 229-33 (1937); J. N. Cumings, *ibid.* 33, 642-4 (1939); J. Scudder, C. R. Drew, D. R. Corcoran, D. C. Bull, *J. Am. Med. Assn.* 112, 2263-71 (1939); John E. Harris, *ibid.* 136, 619-27 (1940); Rubens Salomé Perlera, *J. Biol. Chem.* 160, 617-29 (1945).

[85] Joseph M. Looney and Cora G. Dyer, *J. Lab. Clin. Med.* 28, 355-63 (1942).

[86] P. A. Kometiani and Shushana V. Dolidze, *Biokhimiya* 11, 29-32 (1946).

[87] Earl H. Wood, *J. Lab. Clin. Med.* 27, 960-5 (1942).

precipitate to become colloidal,[88] although the use of ethanol or acetone as a precipitation medium has not been found to improve the sensitivity of the reaction.

As in the sodium cobaltinitrite method, lowering the temperature increases the sensitivity from a minimum of 0.01 mg. per ml., detectable at room temperature, to 0.002 mg. per ml. of potassium determinable at 0°. The reaction is also completed in a briefer period of time.[89] The optimum precipitation temperature is 20° when practicable because at that temperature the nitrite:potassium ratio remains most constant over the widest range.

Procedure.[90] Prepare a silver cobaltinitrite reagent containing 1 per cent of silver nitrate by dissolving 25 grams of sodium cobaltinitrite in 150 ml. of 33 per cent sodium nitrite solution. To this, add with stirring 5 ml. of 40 per cent silver nitrate solution and dilute to 200 ml. Add 2 ml. of glacial acetic acid and pass air through the cold solution for 5 minutes. Allow to stand for 12 hours at 4-6° and filter through a hard filter paper. The solution will keep for two weeks at 4-6°. Centrifuge before use and use the supernatant liquid.

Transfer an aliquot of sample containing 0.1-0.5 mg of potassium to a 15-ml. centrifuge tube and dilute or concentrate to a volume of 1 ml. No acidity stronger than that of acetic acid is permissible. Add 1 ml. of the silver cobaltinitrite reagent, mix, and allow to stand for 3 hours at 4-6°. Then centrifuge at 3000 rpm. for 15 minutes and carefully remove the supernatant liquid. Wash according to the same technic with a 5-ml. portion of water, then with a 5-ml. portion of 60 per cent acetone, and then with portions of 99.5 per cent acetone. A wash solution consisting of 2:1:2 parts of ethanol, ether, and water may be substituted.[91] The precipitate so obtained may be developed in various ways.

Development by Sulfanilic Acid and α-Naphthylamine Dissolve the precipitate in 1 ml. of 0.4 per cent sodium hydroxide solution,[92] immersing the tube in a boiling water bath for 10-15 minutes to hasten solution, and cool. Add 5 ml. of the reagent (page 561) and dilute to a suitable

[88] L. L. Burgess and Oliver Kamm, *J. Am. Chem. Soc.* 34, 652-9 (1912).

[89] Rex J. Robinson and Garth L. Putnam, *Ind. Eng. Chem., Anal. Ed.* 8, 211-13 (1936).

[90] *Ibid.*

[91] John E. Harris, *J. Biol. Chem.* 136, 619-27 (1940).

[92] A. H. Lewis and F. B. Marmoy, *J. Soc. Chem. Ind.* 52, 177-82T (1933).

known volume for development of the color due to the nitrite. Allow the color to develop for 20 minutes and compare with identically treated standards, using a blue filter.

Development by Dimethylglyoxime and Benzidine. To develop this color as cobalt, add 0.1 ml. of concentrated hydrochloric acid to the precipitate and place in an oven at 150°. When dry, cool and take up in 1 ml. of water by heating in a water bath. Cool and add 0.5 ml. of a 1 per cent solution of dimethylglyoxime in ethanol. Add 0.2 ml. of a 1 per cent solution of benzidine in ethanol. Mix well and let stand for 20 minutes before reading the transmittance. Alternatively, balance against a standard prepared at the same time. The color conforms to Beer's law.

Potassium by Dipicrylamine

The reagent known as dipicrylamine is hexanitrodiphenylamine. Potassium forms a stable yellow to orange-red dipicrylaminate[93] which may be used for direct colorimetric estimation in aqueous, acetone,[94] or ethanol solution.[95] When magnesium dipicrylaminate is added to a solution of potassium salt, it loses color in proportion to the amount of potassium dipicrylaminate precipitated from solution,[96] thus providing an indirect method. The sodium salt of dipicrylamine was first used as the precipitating agent, but the lithium salt has been found to have greater precision, in addition to preventing the magnesium or sodium ion concentration from increasing to the point where coprecipitation takes place.[97] The lithium compound is also a desirable form of the reagent because it is improbable that lithium compounds will be present in the sample. The precipitation may take place at 0°,[98] but, by saturating both reagent and wash liquid at room temperature with potassium dipicrylaminate, the determination is carried out more conveniently at room temperature.

Since the aqueous solutions deviate very widely from Beer's law, it is essential as alternatives that (1) a photometric method be used, (2) the standard used for balancing be very close in composition to the sam-

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93 N. S. Poluektov, *Mikrochemie* 14, 265-6 (1934).

94 A. Winkel and H. Maas, *Angew. Chem.* 49, 827-30 (1936); Jacob Kielland, *Ber.* 71B, 220-6 (1938).

95 C. R. Harington, *Biochem. J.* 35, 545-50 (1941).

96 Köhn, *Kali* 35, 108-10 (1941); *ibid.* 36, 65-8 (1942).

97 Elias Amdur, *Ind. Eng. Chem., Anal. Ed.* 12, 731-4 (1940).

98 I. M. Kolthoff and Gordon H. Bendix, *ibid.* 11, 94-8 (1939).

ple, or (3) that a series of standards be used. Although not absolutely essential, accuracy will be improved in balancing instruments, such as the Duboscq, by use of a filter similar to those found desirable for photometric methods. For photometric reading, a sharp cutoff is needed at around 530 mμ. A combination of Corning filter 556, 5.5 mm. thick and Corning 429, 3.8 mm. thick, is useful up to 0.05 mg. per liter.[99] The curve shows closer adherence to Beer's law than with a single filter. As a single filter, 556 mμ gives a nearly straight line. Photometrically a band at 470 mμ is suitable.

Ammonium, rubidium, and cesium salts form similar compounds, although potassium can be determined in the presence of cesium.[100] Beryllium, zirconium, lead, mercuric, and thallous ions form colored crystalline precipitates, and aluminum, nickel, cobalt, copper, bismuth, vanadium, titanium, thorium, ferric, chromic, and mercurous ions form amorphous precipitates with the reagent.[101] If the solution is made slightly alkaline, interference from the latter group of elements is eliminated, and the slight hydrolysis of the potassium complex is suppressed. The complex is fairly stable to variations of temperature. If phosphate is present, the magnesium dipicrylaminate must not be used. Accurate estimation can be made of 0.15-0.8 mg. in the quantity taken for analysis. By increasing the amount of reagent, as much as 80 mg. have been determined, and, by diluting the reagent, quantities smaller than 0.15 mg. may be determined. An accuracy of ±0.5 per cent is possible for the range 1-7 mg. of potassium oxide in 5 ml. of solution.[102]

Procedure.[103] *By Lithium Dipicrylaminate.* Prepare potassium dipicrylaminate by addition of the calculated amount of a 3 per cent solution of the reagent to a potassium chloride solution. Filter the precipitate and wash it with distilled water.

Prepare a 0.6 per cent solution of lithium dipicrylaminate as reagent. First dissolve 0.55 gram of lithium carbonate in 100 ml. of water. To this add 3 grams of dipicrylamine and heat to 50°. Allow to stand for 24 hours, filter into a 500-ml. flask, and dilute to volume. Heat to 50°, and add moist potassium dipicrylaminate until no more dissolves. Let cool to room temperature when some crystals will deposit.

Transfer a sample containing 0.2-0.8 mg. of potassium to a Pyrex

[99] Elias Amdur, *ibid.* 12, 731-4 (1940).
[100] C. J. van Nieuwenburg and T. van der Hoek, *Mikrochemie* 18, 175-8 (1935).
[101] O. G. Sheintzis, *Zavodskaya Lab.* 4, 1047-52 (1935).
[102] Jacob Kielland, *Ber.* 71B, 220-6 (1938).
[103] Elias Amdur, *Ind. Eng. Chem., Anal. Ed.* 12, 731-4 (1940).

tube. If it is necessary to use a sample containing under 0.15 mg. of potassium, add 0.5 mg. of potassinm to hring it into a sensitive range for reading. Evaporate to dryness in an oven. Let cool and add 1 ml. of freshly filtered reagent. Mix well by rotating the tube hetween the hands. Allow to stand for 2 hours at constant temperature. Withdraw 0.4 ml. of clear supernatant liquid by means of a pipet whose tip is covered with filter paper and dilute volumetrically to 100 ml. Compare with a simultaneously prepared standard or series of standards or read photometrically. If the sample contains more than twice as much sodium as potassium, a similar amount of sodium within 10 per cent is necessary in the standard.

By Magnesium Dipicrylaminate. Mix 1.3 grams of magnesium oxide, 3 grams of dipicrylamine, and 100 ml. of water at room temperature. After not less than 24 hours, filter the clear solution for use as reagent.

Evaporate a sample containing 0.1-1.0 mg. of potassium to dryness in a centrifuge tube. Add 1 ml. of reagent and chill in an ice hath. After 15 minutes or more, centrifuge and decant the clear upper layer. Add 1 ml. of ice water, centrifuge, and decant this wash water. Next wash the precipitate with 5 ml. of water saturated with potassium dipicramylaminate, prepared as described in the previous technic. Dissolve the precipitate in about 25 ml. of acetone and dilute to a suitable volume with 0.001 N sodium hydroxide. Read the transmittance of the solution.

MISCELLANEOUS

By precipitation of potassium picrate in alcoholic solution at 20° and suhsequent solution of the precipitate in water, a yellow solution is ohtained suitable for colorimetric estimation of potassium.[104] Below 20° some picric acid is precipitated and above that temperature the solubility of potassium picrate causes low results. Sulfates must be eliminated by precipitation with barinm chloride. Sodium must not exceed 20 mg. in the aliquot and ammonium salts must be absent. Rubidium and cesium interfere, whereas calcium, magnesium, aluminum, iron, phosphates, and silica do not.[105] Small amounts of insoluble matter may be present.

Evaporate the solution containing potassium as the chloride to dryness on a water bath. Dissolve the residne in 1 ml. of water and add,

[104] Earle R. Caley, *J. Am. Chem. Soc.* **53**, 539-45 (1931).

[105] I. N. Antipov-Karataev and A. M. Myasnikova, *Proc. Leningrad Dept. Inst. Fert.* **17**, 81-8 (1933).

with stirring, 7.5 ml. of a saturated solution of picric acid in alcohol. Maintain the temperature at 20° and stir until a precipitate starts to form. Let the solution stand for 45 minutes, stirring every 5 minutes. Filter on an inorganic filter. Transfer the precipitate to the filter with ether and wash with ether until the washings are colorless. No yellow color of picric acid should be visible on the filter or beaker. Usually 5 to 10 washings will take care of this. Continue the suction until ether has been evaporated. Dissolve the precipitate in successive small portions of water until all the precipitate is dissolved, catching the solution in a 50-ml. volumetric flask. After dilution to volume and mixing, compare with a standard potassium picrate solution prepared by similar treatment of a potassium chloride solution. Molybdenum blue may also be employed to determine potassium colorimetrically.[106]

[106] Th. Arnd and E. Leisen, *Bodenkunde u. Pflanzenernähr.* 30, 51-62 (1942).

CHAPTER 38

SODIUM

THE WIDE distribution of sodium in nature leads to equally wide derivation of samples. Thus they may be minerals or water, or almost any form of plant or animal matter. Because so few of its compounds are either insoluble or colored, the methods of estimation are restricted. The primary method for its determination depends on the formation of a triple complex salt with zinc uranyl acetate, followed by indirect determination of sodium by the uranium content of the precipitate. A parallel method as the manganese uranyl acetate complex is probably more sensitive as the manganese can be converted to permanganate, and the corresponding manganese uranyl acetate is more readily precipitated. There is also a corresponding magnesium compound. Other methods have received little attention in recent years.

SAMPLES

Siliceous Material.[1] Boil 10 grams of sample with 20 ml. of 1:1 hydrochloric acid for exactly 2 minutes. Cool somewhat, filter, and use for the determination of sodium, preferably by the nephelometric magnesium uranyl acetate method, omitting the addition of hydrochloric acid.

Water and Soil Extracts.[2] Use an aliquot to contain not over 1 mg. of sodium and evaporate substantially to dryness before addition of reagent. If more than 0.1 gram per liter of potassium is present, results for sodium will be high.[3] Remove excessive amounts of potassium by precipitation with ammonium perchlorate as described under plant material (page 572) starting at "Filter, and to an aliquot . . ." Similarly, if large amounts of lithium are present remove by precipitation with ammonium fluoride.

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[1] F. K. Lindsay, D. G. Braithwaite and J. S. D'Amico, *Ind. Eng. Chem., Anal. Ed.* 18, 101-2 (1946).

[2] D. R. McCormick and W. E. Carlson, *Chemist-Analyst* 31, 15 (1942); B. T. Mulwani and A. G. Pollard, *J. Soc. Chem. Ind.* 56, 128 9T (1937).

[3] C. Sumuleanu and M. Botezatu, *Z. anal. Chem.* 21, 68-74 (1936); D. R. McCormick and W. E. Carlson, *Chemist-Analyst* 31, 15 (1942).

Blood. Place 0.1-0.2 ml. of blood in a platinum dish in an oven at 110° and dry. Set this on a thin layer of sand in a porcelain or metal dish and heat with a low flame until fumes are no longer given off. Cover with a porcelain or quartz plate and heat with a full flame until completely ashed. When cool, dissolve in 0.5 ml. of 1:100 hydrochloric acid and render slightly alkaline by the addition of 0.6 per cent potassium hydroxide solution drop by drop.

Serum.[4] To 0.2 ml. of serum in a 15-ml. conical Pyrex centrifuge tube, add 0.2 ml. of 1:5 sulfuric acid and 0.1 ml. of concentrated nitric acid. Place in a boiling water bath for 10 minutes to diminish foaming during wet ashing and to help hydrolyze the protein. Heat over a free flame with constant shaking to evaporate the water and nitric acid, allowing the flame to strike just above the surface of the liquid. Char, then cool for 30 seconds. Add 1 drop of 30 per cent hydrogen peroxide, heat carefully to fumes of sulfur trioxide, and repeat the addition of peroxide 5 times, heating after each drop. To the cooled colorless solution add 0.9 ml. of water and use as sample.

Alternatively, add 3.5 ml. of water and 1 ml. of 20 per cent trichloroacetic acid solution to 0.5 ml. of serum, mix well, and centrifuge at 3000 rpm. for 10 minutes. Pipet the clear supernatant liquid and recentrifuge if necessary. Trichloroacetic acid filtrates of samples gave values that averaged 2.3 per cent higher than those for ashed samples.[5]

Urine. Measure 6 ml. as sample. Add 1 drop of phenolphthalein solution and 0.2 gram of hydrated lime. The latter precipitates phosphates. If proteins are present, add 0.05 gram of mercuric chloride. Shake and let stand for 30 minutes with occasional shaking. The solution should turn pink with excess lime Filter and collect the filtrate in a test tube. If protein was present, test the filtrate to insure complete precipitation, and if necessary add more mercuric chloride and filter again. Acidify the solution with 1:1 acetic acid.

Milk, Feces, or Organs. Boil 2 ml. or a 2-gram sample with 5 ml. of fuming nitric acid over a small flame until the acid is completely

[4] William S. Hoffman and Bess Osgood, *J. Biol. Chem.* **124**, 347-57 (1938); E. C. Noyons, *Pharm. Weekblad* **76**, 307-11 (1939); M. C. Darnell, Jr. and B. S. Walker, *Ind. Eng. Chem., Anal. Ed.* **12**, 242-4 (1940); A. D. Marenzi and F. Vilallonga, *Anales farm. bioquím.* (Buenos Aires) **11**, 63 9 (1940).

[5] M. C. Darnell, Jr. and B. S. Walker, *Ind. Eng. Chem., Anal. Ed.* **12**, 242-4 (1940).

volatilized. Dissolve the residue in 2 ml. of water and 1 ml. of 1:7 nitric acid. If not completely soluble, add concentrated nitric acid and ash again. Then dissolve in 2 ml. of water and 1 ml. of 1:7 nitric acid. If the iron or phosphorus content is high, add 1 drop of methyl orange solution and 5 drops of a 4 per cent bismuth nitrate solution. Add 50 per cent potassium carbonate solution dropwise until the color changes. Dilute to 10 ml. Centrifuge or allow the precipitate of iron and phosphates to settle. Pipet out 5 ml. of the clear supernatant layer, corresponding to 1 ml. or 1 gram of sample.

Food.[6] Ignite 3-5 grams of sample, first over a low flame, then at higher heat. When all carbon has disappeared, cool and dissolve the residue in 10 ml. of 1:1 hydrochloric acid. Filter if necessary. Dilute quantitatively to 100 ml., mix thoroughly, and use an aliquot containing 2 mg. for the determination of sodium.

Plant Materials.[7] Ash 2-5 grams of air-dried material in a silica dish in a muffle furnace at 700°. Cool, dissolve with 10 ml. of 1:4 hydrochloric acid, and evaporate nearly to dryness. Allow to cool, wash into a 50-ml. flask, and make nearly neutral with 1:1 ammonium hydroxide. Add 20 ml. of a saturated calcium hydroxide solution and dilute to 50 ml. Filter and, to an aliquot of the filtrate containing 0.075-0.200 mg. of sodium, add 3 ml. of saturated ammonium perchlorate solution and 1 drop of concentrated hydrochloric acid, and evaporate to dryness. Extract the residue with five 2-ml. portions of 95 per cent ethanol containing 0.3 per cent of perchloric acid, decanting through a fritted glass crucible after each addition. Evaporate the combined extracts to a small volume and use as sample.

The sulfur as sulfate, calcinm, magnesium, sodium, and potassium are isolated as solution C in determination of lead (page 30). Take an aliquot for determination of sodium.

Removal of Phosphate Ion with Zinc Carbonate.[8] Take a 10-15 ml. aliquot of solution containing 2-8 mg. of sodium. If too much hydrochloric acid is present, remove it by evaporation to dryness. Otherwise the violent reaction may cause loss of material, and too much zinc chlor-

[6] N. V. Tatarinova, *Doklady Vsesoyuz. Akad. Sel'sko-Khoz. Nauk im. Lenina* **1940**, No. 4, 46-8.

[7] Erich Stolze, *Bodenkunde u. Pflanzenernähr.* 8, 217-25 (1938).

[8] O. R. Overman and O. F. Garrett, *Ind. Eng. Chem., Anal. Ed.* 9, 72-3 (1937).

ide will go into solution. To the dry residue add 10 ml. of water and just enough 1:2 hydrochloric acid to bring all the salts into solution.

Add an excess of powdered zinc carbonate, cover, and let stand at room temperature at least 6 hours or overnight. Filter and wash 6 times with cold water. Combine the filtrates and use to determine sodium, preferably by one of the uranyl acetate methods.

Standard

Dissolve 0.2541 gram of dry, recrystallized sodium chloride in water and dilute to 1 liter. Each ml. will contain 1 mg. of sodium. For a standard containing 0.1 mg. per ml. dilute 10 ml. to 100 ml.

Sodium as the Zinc Uranyl Acetate Complex

Under controlled conditions of acidity sodium is precipitated as a hydrated triple salt, the zinc uranyl acetate complex.[9] $[UO_2(CH_3COO)_2]_3$, $Zn(CH_3COO)_2$, $NaCH_3COO \cdot 9H_2O$, and the uranium determined colorimetrically or photometrically.[10] The other component in the complex can also be manganese or magnesium, for the development of which separate methods follow. The zinc complex method has found widest application.

The presence of calcium, magnesium, aluminum, iron, and manganese does not interfere, but if 4 times as much potassium as sodium is present, treat initially with perchloric acid or ammonium perchlorate. Protein may be removed with mercuric chloride.[11] Arsenate and phosphate interfere. Several methods are applied to the removal of phosphorus, such as precipitation with magnesia mixture, with powdered calcium hydroxide, with lead acetate and subsequent removal of excess lead with magnesium sulfate, with basic zinc acetate in 50 per cent ethanol, with barium chloride and subsequent removal of excess barium with ammonium carbonate, or with zinc carbonate[12] With the exception of the last, these reagents tend to leave in solution a high salt concentration which may crystallize out if the volume of solution is reduced. Use of zinc carbonate avoids this.

The precipitate of the uranyl acetate complex should be allowed to

[9] H. H. Barber and I. M. Kolthoff, *J. Am. Chem. Soc.* 50, 1625-31 (1928); 51, 3233-7 (1929).

[10] A. D. Marenzi and F. Vilallonga, *Anales farm bioquím.* (Buenos Aires) 11, 63-9 (1940); James T. Bradbury, *J. Lab. Clin. Med.* 31, 1257-61 (1946).

[11] B. T. Mulwani, *J. Univ. Bombay* 8, Pt. 5, 128-34 (1940).

[12] O. R. Overman and O. F. Garrett, *Ind. Eng. Chem., Anal. Ed.* 9, 72-3 (1937).

stand at 0° for 1 hour.[13] The aqueous solution of sodium uranyl zinc acetate subsequently formed is read directly with a blue filter around 400-450 mμ.[14] The optimum amount of sodium in the aliquot of sample is about 0.3 mg. When the sodium content is very critical, such as in blood serum where small deviations may be of pathological significance, it is advisable to determine sodium photoelectrically so that the minimum experimental error may be present. The color produced by aqueous solutions is subject to variations due to even small temperature changes. The complex has a tendency to decompose, especially in sunlight or on heating, and form a deeper-hued precipitate. This is probably due to a disturbed equilibrium between hexavalent uranium ion and the more highly colored uranyl salt molecules.[15] Washing the precipitate with ethanol or acetone has a solubilizing effect. Ethanol-acetic acid and ethyl acetate-acetic acid mixtures are sometimes so used.

The sensitivity of measurement of aqueous solutions of the triple acetate is limited. Rather than attempt to control temperature or to apply temperature corrections, several reagents are used to dissolve the precipitate to stabilize the color against temperature changes and to secure a sensitive coloration. The aqueous solution of the triple acetate when treated with potassium ferrocyanide[16] forms the stable brownish red potassium uranyl ferrocyanide complex, $UO_2K_2Fe(CN)_6$. The intensity of the color is subject to variation with temperature, pH, and concentration of reagents. Large additions of potassium ferrocyanide increase the color and delay precipitation.

Ammonium thiocyanate[17] gives a yellow color which may be determined photoelectrically with an accuracy of 1-2 per cent. If a solution of hydrogen peroxide, diluted from the 30 per cent grade, is added to a solution of uranyl salts in sodium or ammonium carbonate solution, an intense yellow to red solution results, the color depending upon the concentration of the uranyl ion.[18] The usual stabilizers, such as acetanilide, in commercial 3 per cent hydrogen peroxide render it unsatisfactory

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[13] E. C. Noyons, *Pharm. Weekblad* 76, 307-11 (1939).

[14] William S. Hoffman and Bess Osgood, *J. Biol. Chem.* 124, 347-57 (1938); L. Jendrassik and M. Holász, *Biochem. Z.* 298, 74-80 (1938); D. R. McCormick and W. E. Carlson, *Chemist-Analyst* 31, 15 (1942).

[15] William S. Hoffman and Bess Osgood, *J. Biol. Chem.* 124, 347-57 (1938).

[16] B. T. Mulwani and A. G. Pollard, *J. Soc. Chem. Ind.* 56, 129 9T (1937); B. T. Mulwani, *J. Univ. Bombay* 8, 128-34 (1940).

[17] W. S. Hoffman and B. Osgood, *J. Biol. Chem.* 124, 347-57 (1938); R. F. Reitmeier, *Ind. Eng. Chem., Anal. Ed.* 15, 397 (1943).

[18] Eric A. Arnold and Alfred R. Pray, *Ind. Eng. Chem., Anal. Ed.* 15, 294 6 (1943).

for this use. This color is dependent upon the concentration of reagents and is desirably read at 520 mμ with a spectrophotometer.

Sodium salts of phenolic acids develop colors with solutions of uranyl salts. As an example, sulfosalicylic acid forms an orange color with the uranyl acetate complex, which is stable for 3 hours but subject to variation with temperature and acidity changes.[19] A filter which exhibits maximum transmission at 440 mμ is suitable. The color does not conform exactly with Beer's law, but reproducible results can be achieved. Greater sensitivity is obtainable by adjustment of the reagent to approximately the phenolphthalein end point, but the more intense color so obtained is more sensitive to variations in acidity and does not conform as closely to Beer's law as under the conditions selected.

When color is developed by addition of an alcoholic solution of alizarin, the accuracy is to 0.002 mg. in 0.025-0.120 mg. of sodium.[20] Pyrocatechol and sodium hydroxide serve as another medium in which the uranyl complex may be dissolved and sodium determined.[21]

Procedure. Transfer an aliquot of sample solution containing 0.05-0.3 mg. of sodium to a 12-ml. centrifuge tube. Evaporate in a water bath to 0.2 ml. and cool.

Prepare a uranyl zinc acetate reagent by mixing 80 grams of uranyl acetate dihydrate with a solution containing 14 ml. of glacial acetic acid in 427 ml. of water. Into another solution of 7 ml. of glacial acetic acid in 294 ml. of water, stir 220 grams of zinc acetate dihydrate. Heat the 2 solutions separately on a water bath to dissolve. Mix while hot, cool, and add 0.2 gram of sodium uranyl zinc acetate crystals. To obtain these crystals, add 125 ml. of the uranyl zinc acetate reagent to 5 ml. of a 2 per cent sodium chloride solution and filter through a porous porcelain crucible. Wash several times with glacial acetic acid and then with ether, and dry in a desiccator over calcium chloride for 1 hour. Allow the uranyl zinc acetate reagent to stand overnight, store in a dark bottle, and filter immediately before use.

To the solution of sample add 8 ml. of uranyl zinc acetate reagent and mix by inversion for 1 minute. Let stand for 1 hour at 0° Centrifuge for 10 minutes at 3000 rpm. and drain over filter paper for 10 minutes. Wipe the mouth of the tube with filter paper. Wash the precipitate with a 1:5 glacial acetic acid-ethanol solution saturated with

[19] M. C. Darnell, Jr. and B. S. Walker, *ibid.* **12,** 242-4 (1940).
[20] Eugène Fredericq, *Bull. soc. chim. Belg.* **51,** 199 208 (1942).
[21] C. Sumuleanu and M. Botezatu, *Z. anal. Chem.* **21,** 68 74 (1936).

sodium uranyl zinc acetate.[22] Centrifuge at 3000 rpm. for 10 minutes, decant, and drain for 10 minutes. Wipe the mouth of the tube and wash with two 5-ml. portions of ether to remove all traces of acid, centrifuging after each addition for 5 minutes, and decanting. Allow the tube to remain unstoppered to evaporate the ether completely.

Alternatively, to 1 ml. of sample add 5 ml. of freshly filtered reagent. Mix well and let stand. At 5-minute intervals, add seven 0.3-ml. portions of 95 per cent ethanol. Mix after the first five additions by rolling the tube between the hands. Wash down the sides of the tube with the last two additions and let them layer on the surface. Finally centrifuge at 2000 rpm. for 10 minutes, decant, invert, and drain for 5 minutes. Wipe the mouth of the tube dry and agitate the precipitate with 2 ml. of a mixture of 30 ml. of ethyl acetate diluted to 100 ml. with glacial acetic acid. Wash down the wall of the tube, centrifuge, and complete as before. Wash tho precipitate and the wall of the tube with 5 ml. of ether. This time in completing, drain for only 1 minute, as the precipitate may otherwise drop out. Repeat the ether wash and evaporate the last traces of ether by putting in a warm place for 5 minutes.

These precipitates are dissolved and the color developed in various ways.

Direct Reading. Add water at 60-70° in 2-ml. portions until the precipitate is dissolved, collecting the solution in a comparison tube. When cool, dilute to a standard volume according to the intensity of color. Compare with a standard prepared by similar treatment, or read the transmittance at 400-465 mμ and compare with a calibration curve.

By Potassium Ferrocyanide. Dissolve the precipitate in water and transfer to a 25-ml. flask. Add 1 drop of glacial acetic acid and 0.5 ml. of a 20 per cent potassium ferrocyanide solution. Dilute to 25 ml., shake for 1 minute, and compare with simultaneously prepared standards. Alternatively, read the transmittance and compare with a calibration curve.

By Ammonium Thiocyanate. To the precipitate add 10 ml. of a solution of ammonium thiocyanate, prepared by dissolving 3.81 grams of salt, and diluting to 500 ml. Mix to dissolve the precipitate and centrifuge at 3000 rpm. for 5 minutes to remove any phosphate precipitate. Read tbe transmittance of the clear upper layer, using a blue filter, and compare with a calibration curve.

[22] Eric G. Ball and Joseph F. Sadusk, Jr. *J. Biol. Chem.* 113, 661-74 (1936).

By Sulfosalicylic Acid. Transfer the precipitate quantitatively to a 100-ml. volumetric flask, using 4-5 ml. of water. Add to the contents of the flask 65 ml. of water, 4 ml. of a 5 per cent sulfosalicylic acid solution, and 4 ml. of a 10 per cent sodium acetate trihydrate solution. Dilute to volume, mix thoroughly, and read against a calibration curve, using a 440-mμ filter.

By Hydrogen Peroxide. Dissolve the triple acetate in 10 ml. of water and add 6 ml. of a saturated solution of ammonium carbonate. Add 6 ml. of 3 per cent hydrogen peroxide prepared by 1:10 dilution of the 30 per cent grade and mix. Dilute to 25 ml. and read immediately against simultaneously prepared standards or read the transmittance at 460-520 mμ against a calibration curve.

By Alizarin. Prepare a reagent by dissolving 2 grams of alizarin per liter of 95 per cent ethanol. Store this at 40° for 24 hours and filter. Dissolve the precipitate in water and dilute to about 90 ml. in a 100-ml. volumetric flask. Add 7 ml. of the reagent solution and dilute to volume. Read the transmittance with a 570-mμ filter. The zero setting on the instrument is taken with 7 ml. of reagent and 40 ml. of 95 per cent ethanol diluted to 100 ml. The plot obtained is a straight line, showing that Beer's law applies, but does not pass through the origin because the reagent itself is colored.

Sodium as the Manganese Uranyl Acetate Complex

Sodium may be precipitated as the complex manganous sodium uranyl acetate, and the sodium determined by reading the permanganate color obtained on oxidation of the complex with potassium periodate.[23] Phosphate should be removed unless the concentration is less than 10 per cent of the sodium concentration. In the case of organic material such as blood serum or urine, removal of phosphate is not necessary.

Potassium causes results that are high if present in a ratio of more than 1.5 times the sodium concentration. A correction may be made for its presence, or it may be removed by precipitation as the perchlorate. The optimum concentration for determination by this method is 0.03-0.12 mg. of sodium, although a much wider range is determined by varying the concentration of reagents

[23] H. H. Willard and L. H. Greathouse, *J. Am. Chem. Soc.* 39, 2366 77 (1917); Warren C. Woelfel, *J. Biol. Chem.* 125, 219 27 (1938).

Washing the precipitate with glacial acetic acid [24] or alcohol-ether mixtures is unsatisfactory.[25] A 25 per cent alcoholic solution of zinc uranyl acetate saturated with sodium manganous uranyl acetate is recommended. The reagent is prepared in water solution and mixed with alcohol only when it is ready for use, to prevent reduction of the uranyl ion by the alcohol.

Procedure. Prepare a reagent by adding 16 grams of uranyl acetate dihydrate, 49 grams of pure manganese acetate tetrahydrate, and 13.8 ml. of 30 per cent acetic acid to 129.2 ml. of water, making about 200 ml. of solution. Store in a brown bottle. When ready to use, prepare a 25 per cent alcoholic solution of manganous uranyl acetate by adding 3 ml. of 95 per cent ethanol to a 9-ml. portion of reagent, mix, and cover. Allow to stand in the dark for 4 hours and filter. This alcoholic mixture may be safely stored in the dark for 3 weeks.

Transfer a 1-ml. aliquot of protein-free sample containing 0.02-0.15 mg. of sodium into a 15-ml. conical centrifuge tube. Add 9 ml. of the 25 per cent alcoholic solution of manganous uranyl acetate and mix well with a rod. The precipitate should appear within 1 minute. Wash the rod with 1 ml. of reagent. Cover the tube with a rubber cap and allow it to stand for 4-5 hours. Centrifuge the covered tube and remove the supernatant liquid.

Prepare a 25 per cent alcoholic zinc uranyl acetate wash solution as follows. Add 16 grams of uranyl acetate dihydrate, 44 grams of zinc acetate tetrahydrate, and 13.8 ml. of 30 per cent acetic acid to 134.2 ml. of water to make about 200 ml. of solution. When ready to use, prepare from it a 25 per cent alcoholic wash solution by adding 12 ml. of zinc uranyl acetate solution to 4 ml. of ethanol. Saturate the solution with sodium manganous uranyl acetate. The salt is prepared by adding 125 ml. of manganous uranyl acetate stock solution to 2 ml. of a 5 per cent sodium chloride solution, stirring, and allowing to stand 0.5 hour for the precipitate to form. Remove the supernatant liquid, centrifuge the precipitate, and again remove the supernatant liquid. Wash with three 5-ml. portions of 95 per cent ethanol and two 5-ml. portions of ether, mixing the solvent with the precipitate, and centrifuging after each addition. Allow the ether to evaporate and store the sodium manganous uranyl acetate in a brown vial. Ordinarily 20 mg. of the triple salt will saturate 100 ml. of the wash solution. Allow the saturated wash solution

[24] Warren C. Woelfel, *J. Biol. Chem.* **125**, 219-27 (1938).
[25] Ernst Leva, *ibid.* **132**, 487-99 (1940).

to stand for 1 hour with occasional shaking and filter. The solution should keep for 3 weeks if stored in the dark.

Pipet into the precipitate three 4-ml. portions of the above wash solution, stirring, centrifuging and removing the supernatant liquid after each addition. Evaporate the alcohol by placing the tube in a water bath under a hood and raising the temperature slowly to boiling. After 15-20 minutes the alcohol should be completely evaporated. If the precipitate is too large, dissolve in water, and take an aliquot portion for subsequent development of color.

Prepare a potassium periodate solution by dissolving 2.5 grams of potassium periodate in a 1:4 dilution of 85 per cent orthophosphoric acid and diluting to 1 liter with that acid. Add 10 ml. of periodate solution to the tube and place in boiling water for 10-15 minutes. Cool, transfer quantitatively to a suitable volumetric flask, and dilute to volume. Compare with a potassium permanganate standard or read the transmittance using a 520-mμ filter.

Sodium as the Magnesium Uranyl Acetate Complex

Magnesium uranyl acetate [26] has been employed for the precipitation of sodium as the complex triple salt, sodium magnesium uranyl acetate.[27] Sodium is determined by reading the color of the precipitate dissolved in water,[28] in aromatic hydroxycarboxylic acids, and in alizarin.[29] Thus the methods are primarily related to the uranium in the complex, the advantage for the magnesium precipitate being greater ease of precipitation. Methods of development of the color given for the zinc uranyl complex are applicable to this one also.

An ethanol-magnesium uranyl acetate reagent [30] is used in a nephelometric procedure for the determination of sodium with an accuracy of 0.006 mg. per ml.[31] Similar determinations with methanol, isopropanol, or acetone reagents are less sensitive, but a mixture of 86 per cent ethanol and 10 per cent methanol is satisfactory. Determination of sodium in aqueous solution with this reagent is made more sensitive by the addition of small quantities of ethanol.[32]

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[26] Earle R. Caley and C. W. Foulk, *J. Am. Chem. Soc.* 51, 1664-74 (1929).

[27] Ernest Kahane, *Bull. soc. chim.* [4], 47, 382-404 (1930).

[28] N. V. Tatarinova, *Doklady Vsesoyuz. Akad. Sel'sko-Khoz. Nauk. im. Lenina* 1940, No. 4, 46-8.

[29] Eugène Fredericq, *Bull. soc. chim. Belg.* 51, 199-208 (1942).

[30] Earle R. Caley, C. T. Brown and H. P. Price, *Ind. Eng. Chem., Anal. Ed.* 6, 202-5 (1934); C. H. Greene, *ibid.* 8, 399-400 (1936).

[31] F. K. Lindsay, D. G. Braithwaite and J. S. D'Amico, *ibid.* 18, 101-2 (1946).

[32] C. H. Greene, *ibid.* 8, 399-400 (1936).

The maximum sodium concentration for the determination is 0.75 mg. per ml. Potassium and lithium do not precipitate if the chloride concentrations are less than 10 and 11 per cent respectively.[33] Arsenates, phosphates, and free mineral acid interfere.

Procedure. *Colorimetric.* To prepare a reagent, dissolve 85 grams of sodium-free uranyl acetate in 60 grams of glacial acetic acid, and 600 grams of magnesium acetate in 60 grams of glacial acetic acid. Dilute each solution to 1 liter. Mix the two solutions, allow to settle, and filter after at least 24 hours.

To an aliquot of sample, containing 0.5-5.0 mg. of sodium, which should be either diluted or evaporated to 1 ml., add 10 ml. of reagent. Keep at 20° for 40 minutes, shaking frequently. Allow the precipitate to settle and decant the supernatant layer. Wash with four 2-ml. portions of 95 per cent ethanol.

Dissolve the precipitate in water at 60-70° and dilute volumetrically to 50 ml. Compare colorimetrically with a similarly prepared standard. For greater sensitivity develop the color by one of the methods given for the zinc uranyl complex.

Nephelometric. To prepare the reagent solution, mix 500 ml. of 95 per cent ethanol, 30 grams of uranyl acetate dihydrate, 150 grams of magnesium acetate tetrahydrate and 20 ml. of glacial acetic acid. Dilute to 1 liter. Warm on a steam bath with stirring to dissolve salts, permitting as little of the solvent as possible to evaporate. Stir until cool and filter into a brown glass bottle.

To a 2-ml. aliquot of sample solution, add 1 drop of concentrated hydrochloric acid and 15 ml. of the alcoholic reagent. More alcohol will cause the precipitate to form too rapidly and decrease the accuracy of measurement. Mix by inverting 5 times, let stand for 5 minutes, and again invert 5 times. After 5 minutes, read the transmittance or compare against similarly prepared standards.

Sodium Separated as Sodium Cesium Bismuth Nitrite

Sodium may be separated from potassium and other metals with which it is commonly associated by precipitation as the complex sodium cesium bismuth nitrite, $6NaNO_2 \cdot 9CsNO_2 \cdot 5Bi(NO_2)_3$.[34] This salt may be dissolved and sodium indirectly determined by the bright red color pro-

[33] A. Elías, *Anales asoc. quím. Argentine* **23**, 1-3 (1935).
[34] W. C. Ball, *J. Chem. Soc.* **97**, 1408 (1910).

duced by nitrites with sulfanilic acid and α-naphthylamine.[35] Chlorides should not be present in concentrations greater than 0.7 per cent or bismuth oxychloride may separate. More than traces of phosphates should be removed. Other interfering substances are iodides, citrates, and most of the heavy metals, particularly iron and silver. The use of potassium-glass containers is recommended. Another indirect method is to estimate bismuth as the colloidal sulfide.[36]

Procedure. Dissolve 30 grams of sodium-free potassium nitrite in about 60 ml. of water. Add 3 grams of bismuth nitrate dissolved in 5 ml. of 1:7 nitric acid. If a precipitate forms, add 1:1 nitric acid until it redissolves. Add 16 ml. of a 10 per cent solution of cesium nitrate. Dilute to 100 ml. and again add 1:1 nitric acid if any turbidity appears. The reagent should be a clear orange-yellow. If sodium salts were present, cool to 0° and filter off the precipitate at the end of 24 hours. This reagent keeps under illuminating gas at 0° for several weeks. If exposed to air, a white scum forms.

Cool the aliquot of sample to 10-12° and add 3 ml. of bismuth cesium nitrite solution for each mg. of sodium expected. Stopper the flask with a 2-hole rubber stopper holding 2 right-angle glass tubes. Attach to one a short rubber tube with a glass plug, and to the other a Bunsen valve and plug. Remove the plugs, pass illuminating gas free from hydrogen sulfide into the flask for a few seconds, and replace the plugs. Cool to 0° for 24 hours, when a yellow crystalline precipitate will have formed. At room temperature it takes 48 hours, and a scum is much more likely to form.

Development of Color with Sulfanilic Acid and α-Naphthylamine. Filter rapidly on a Gooch crucible Wash quickly with five 2-ml. portions of ice cold 50 per cent acetone solution, saturated with sodium cesium bismuth nitrite. Dissolve the precipitate by warming with 10 ml. of an alkaline tartrate mixture containing equal parts of 10 per cent potassium hydroxide solution and 10 per cent tartaric acid solution. Transfer to a 100-ml. volumetric flask, cool, dilute to volume, and mix. Remove an aliquot corresponding to about 0.01 mg. of sodium to another 100-ml. flask. Dilute this and 1 ml. of a standard nitrite solution in a similar flask to about 90 ml. Add to each 2 ml. of an 0.8 per cent solution of sulfanilic acid in 1:3 acetic acid, and 2 ml. of a 0.5 per cent solution of

[35] Edward A. Doisy and Richard D. Bell, *J. Biol. Chem.* **45**, 313-23 (1920).
[36] Ernst Tschopp, *Helvetica Chim. Acta* **8**, 893-900 (1925).

α-naphthylamine in 1:3 acetic acid. Dilute to 100 ml., shake, and compare after allowing 20 minutes for development of the color. As this develops the color from the nitrite present it may be very bright. The average error is about 5 per cent.

As the standard sodium nitrite solution, dissolve 0.0300 gram of pure sodium nitrite in water and dilute to 1 liter. Each ml. contains nitrite equivalent to 0.01 mg. of sodium.

Development of Color with Hydrogen Sulfide. Filter and wash the precipitate exactly as in the preceding method. Dissolve the precipitate in 10 ml. of 1:7 nitric acid, transfer to a 100-ml. volumetric flask, and dilute to volume. Transfer 10 ml. of this to a 25-ml. volumetric flask and add 5 ml. of a 1 per cent gum arabic solution. Dilute to volume with a saturated aqueous solution of hydrogen sulfide. Compare with a standard bismuth solution treated with the same reagents.

As the standard bismuth nitrate solution, dissolve 7.591 grams of pure bismuth in 50 ml. of concentrated nitric acid and dilute to 1 liter. Dilute 10 ml. of this to 1 liter. A 10-ml. portion of the latter contains 0.7591 mg. of bismuth, which corresponds to 0.1 mg. of sodium in the complex compound.

Miscellaneous

An indirect method for sodium is to precipitate it as pyroantimonate, dissolve the sodium pyroantimonate, and determine antimony from the compound in solution as the orange colloidal sulfide.[37] As reagent, dissolve 10 grams of powdered potassium pyroantimonate in 500 ml. of boiling water. Cool rapidly and add 15 ml. of 10 per cent sodium-free potassium hydroxide solution. Stir and filter through ash-free filter paper into a paraffined bottle. The solution keeps a month or longer.

To 1 ml. of potassium pyroantimonate reagent in a centrifuge tube, add the sample and then 0.3 ml. of 95 per cent ethanol dropwise with stirring. Let stand for 45 minutes and centrifuge. Decant the upper layer and wash the precipitate with three 2-ml. portions of 50 per cent ethanol. Dissolve the residue in 0.5 ml. of concentrated hydrochloric acid. Transfer to a 25 ml.-volumetric flask, rinsing the centrifuge tube with 1 ml. of 1:10 hydrochloric acid. Put 3 ml. of a standard solution of sodium pyroantimonate into a second 25-ml. flask. To each add 10 ml. of water, 3 ml. of a 10 per cent gelatin solution, and 2.5 ml. of a 10 per

[37] Shum-ichi Yoshimatsu, *Tôhoku J. Exptl. Med.* 8, 496-500 (1927).

cent solution of sodium sulfide. Shake, dilute to volume, and compare the two solutions in a colorimeter.

To prepare the standard, slowly add 50 ml. of 2.5 per cent sodium chloride solution to 500 ml. of the potassium pyroantimonate reagent. Then add 120 ml. of 95 per cent ethanol with constant stirring. Let stand at least 2 hours, transfer to a wet pad of filter paper on a Büchner funnel, and wash with 300 ml. of 50 per cent ethanol or until the washing fails to show a color on the addition of hydrochloric acid and sodium sulfide. Dissolve 1.108 grams of the dried sodium pyroantimonate in 125 ml. of concentrated hydrochloric acid, and dilute to 1 liter with water. Each ml. corresponds to 0.1 mg. of sodium.

CHAPTER 39

LITHIUM

As one of the minor alkali metals, lithium would not be expected to be widely distributed, and this proves to be the case. On the other hand, it is a tonnage industrial material such as in the form of the hydride, and therefore occasions for its determination necessarily arise. Thus, aside from the conventional sources such as minerals, ceramics, and water, it is present in greases and medicinal products. It is either determined indirectly by a colorimetric method dependent on the separation of an iron double compound or turbidimetrically as the stearate.

Standard

Dissolve 0.6200 grams of lithium chloride, freshly ignited under conditions to avoid hydrolysis, in water and dilute to 100 ml. Each ml. of standard solution will contain 1 mg. of lithium. Alternatively, dissolve 0.532 grams of dried lithium carbonate in 1:100 hydrochloric acid or 0.792 grams of freshly ignited lithium sulfate in water and dilute to 100 ml.

Lithium by Potassium Ferric Periodate

By treatment of a solution of lithium with potassium ferric periodate in alkaline solution, the lithium is precipitated as lithium potassium ferric periodate, $LiKFeIO_6$.[1] It follows that such a precipitate can be redissolved and the varied methods for determination of iron applied as indirect methods for lithium. Calcium and magnesium also precipitate with the reagent and must be absent. Sodium not only forms a compound of corresponding structure and low solubility, but also that compound is strongly coprecipitated with the lithium compound. Fortunately sodium and potassium in excess can be separated. The sodium remaining must be controlled and provided for in the standard curve used.

[1] O. Procke and A. Sloef, *Collection Czechoslaw. Chem. Commun.* 11, 273 (1939); E. B. Sandell, "Colorimetric Determination of Traces of Metals," pp. 301-4. Interscience Publishers, New York, N. Y. (1944).

Procedure. To prepare the reagent add slowly with stirring 12 ml. of a 2.7 per cent solution of ferric chloride hexahydrate in 1:60 hydrochloric acid to 10 ml. of 11.2 per cent potassium hydroxide solution and 40 ml. of a 5.7 per cent solution of potassium paraperiodate. Dilute the mixture to 100 ml. with 11.2 per cent potassium hydroxide solution. Filter if not clear and store in a paraffin-lined bottle.

Take an amount of sample to contain 0.02-0.10 mg. of lithium. Only chlorides may be present as negative radicals. Evaporate to dryness and take up in a minimum volume of water, about 0.3 ml. per 100 mg. of chlorides. To this solution add 8 ml. of absolute ethanol and 20 ml. of absolute ether. Mix well, cover, and let stand for 5 minutes. Filter and wash the residue with 1:4 absolute ethanol-ether mixture. The lithium salts have now been separated from the sodium and potassium. Evaporate the filtrate and washings to dryness and take up in a drop or two of water. Add 1 ml. of 5.6 per cent potassium hydroxide and heat to just below boiling.

Add 2 ml. of the prepared reagent, mix, and maintain just below boiling for 5 minutes with intermittent stirring. Filter on an inorganic filter and wash with four 1-ml. portions of 5.6 per cent potassium hydroxide solution. Dissolve the precipitate in 10 ml. of cold 1:11 hydrochloric acid, using a 25-ml. volumetric flask as receiver, and dilute to volume with water. Use 5-ml. portions for determination of iron by any of the methods applicable to such a solution. Rather than use of the theoretical factor for the ratio of lithium to iron, it is preferable to use a standard curve actually determined with reagents of the same kind and quality as are used in the determination.

As an example of application of the iron methods, to the 5-ml. aliquot add water to dilute to 20 ml. Add 3 ml. of 20 per cent potassium thiocyanate solution, dilute to 25 ml., and read the transmittance.

Lithium by Ammonium Stearate

Lithium stearate, unlike other alkali stearates, is relatively insoluble in organic solvents. This property is the basis of a method for its estimation.[2] The method is accurate to 0.02 mg. in the lower concentrations and to 0.05 mg. in the upper ranges.

Procedure. Concentrate a suitable volume of a hydrochloric acid solution of the sample to a small volume. Add 10 ml. of amyl alcohol and heat on a sand bath until all of the water has been expelled. If nec-

[2] Earle R. Caley, *J. Am. Chem. Soc.* **52**, 2754-8 (1930).

essary, add more amyl alcohol. This operation will be facilitated by passing a slow stream of dry air through the solution. A precipitate of sodium and potassium chlorides separates. Some lithium hydroxide may also separate. Decant the solution of lithium chloride in amyl alcohol through a dry filter. Wash the residue of salts and the filter with 2-ml. portions of hot amyl alcohol.

Moisten the residue of salts with 2 ml. of 1:1 hydrochloric acid, dissolve in 3 ml. of water, and concentrate to a small volume. Repeat the extraction with amyl alcohol. This operation may have to be repeated 4-5 times if much lithium is present. Normally twice will suffice when the amount of lithium is so small as to require the colorimetric method of estimation. Combine the amyl alcohol extracts and dilute to a suitable volume for use as sample. As an alternative, 2-ethyl hexanol can replace the amyl alcohol.[3]

As reagent,[4] dissolve about 20 grams of stearic acid in 1 liter of ether. Pass in ammonia gas until no further precipitation of ammonium stearate takes place. Add ether from time to time to replace that lost by evaporation. Pour the suspeasion into a large tray and let the ether evaporate. Dissolve 2 grams of the ammonium stearate in 100 ml. of warm amyl alcohol. The solvent must not he heated over 50° or the ammonium stearate will be partially decomposed. The concentration specified is near saturation. The solution must be prepared fresh nearly every day as it loses ammonia on standing.

Place 2.0 ml. of the amyl alcohol solution of the sample in a test tube. Prepare others with 0.05, 0.075, 0.1, 0.15, 0 25 and 0.4 mg. of lithium in 2 ml. of amyl alcohol. To the sample and standards, add 5.0 ml. of the ammonium stearate reagent in amyl alcohol. Stopper and shake all at the same time. Let stand for 30 minutes, shake again, and compare.

[3] Earle R. Caley and Herbert D. Axilrod, *Ind. Eng. Chem., Anal. Ed.* **14**, 242-4 (1942).

[4] LeRoy McMaster, *J. Am. Chem. Soc.* **36**, 1918 (1914).

CHAPTER 40

CESIUM

CESIUM is another rare alkaline metal which is not widely distributed. Although it finds substantial use in vacuum tubes and photoelectric cells, little work has been done in developing a colorimetric method for determination of small amounts. It is assumed that the sample is available in aqueous solution.

STANDARD

Dissolve 1.2666 grams of cesium chloride in water and dilute to 1 liter. Each ml. contains 1 mg. of cesium.

CESIUM BY A SPOT REACTION WITH GOLD AND PLATINUM BROMIDES

A mixture of gold and platinum bromides forms a deep black precipitate with a cesium salt, whose composition appears to be $Cs_2Au_2PtBr_{12}$.[1] If the metal is present in minute quantities, the spot appears gray. The coloration is suitable for a colorimetric spot test for cesium by comparison with a series of standards. Rubidium interferes to some extent if its concentration exceeds 2 per cent. Potassium, sodium, lithium, and ammonium chlorides do not influence the reaction. The accuracy of the method is in the range of 5-10 per cent.

Procedure. Prepare a reagent comprising 3 per cent of gold bromide and 1.5 per cent of platinum bromide. Place a drop of the reagent solution on filter paper, and on the spot place a drop of the sample solution. Prepare a series of standards from equal drops of reagent on filter paper treated with suitable dilutions of standard cesium solution. When this scale is dried, it is fairly permanent. Compare the unknown with the series and interpolate the amount of cesium present.

MISCELLANEOUS

Cesium is determined in potassium aluminum sulfate[2] by the molyb-

[1] E. S. Burkser and M. L. Kutschment, *Mikrochemie* 18, 18-21 (1935).
[2] E. S. Burkser and R. V. Feldman, *Zavodskaya Lab.* 7, 166-8 (1938).

denum blue reaction. For use, treat 2 ml. of a solution of the salt in 2:1 hydrochloric acid with 0.2 ml. of 1 per cent sodium silicomolybdate solution. Centrifuge, and wash the silicomolybdate precipitate by decantation with 5 ml. of water. Dissolve with shaking in 30 ml. of 1:15 hydrochloric acid. Reduce with 1 ml. of 5 per cent stannous chloride solution and compare the blue color with similarly prepared standards.

CHAPTER 41

CALCIUM

THE WIDE distribution of calcium in minerals, foods, and body fluids leads to correspondingly frequent need for determination of small amounts. Since it forms no simple colored compounds the methods are either turbidimetric or indirect. Many of the latter are time consuming. Some require double precipitations or expensive reagents, or are subject to interference from many sources. The most widely used precipitant is oxalate, and diverse methods are applied to determination of the amount of oxalate. If precipitated as phosphate, more sensitive methods are applicable to the phosphate radical, but the constancy of ratio of calcium to phosphate is always in doubt. Turbidity methods usually use a calcium soap.

SAMPLES

Minerals. For many determinations of small amounts the sample will have been carried through the classical procedure up to the precipitation of calcium as the oxalate. If the precipitate is insufficient for accurate gravimetric or volumetric determination, dissolve in 0.5 ml. of 1:1 hydrochloric acid for colorimetric estimation.

Limestone. Ignite 0.3 gram of sample in a platinum crucible. If the limestone is argillaceous, add an equal amount of anhydrous sodium carbonate before ignition. Cool, add 2 ml. of water slowly, followed by 2 ml. of 1:1 hydrochloric acid. Stir, crush any lumps, and transfer to a beaker. Add 2 ml. of water and 2 ml. of 1:1 hydrochloric acid to the crucible, warm gently, and transfer to the beaker, rinsing the crucible thoroughly. Heat the beaker to dissolve the contents, except silica. Dilute to about 100 ml. Add a slight excess of bromine water and 10 drops of a 0.1 per cent solution of bromophenol blue. Add 1:1 ammonium hydroxide dropwise with stirring until the solution turns blue, or until precipitation of ferric hydroxide indicates that the solution is nearly neutral. Dilute to a suitable volume for use of aliquots.

[1] Wilbur H. McComas, Jr. and William Rieman III, *Ind. Eng. Chem., Anal. Ed.* 14, 929-31 (1942).

Boiler Scale. The early stages of preparation of the sample has been described under aluminum (page 242). Transfer 12 ml. of the solution so prepared to a 15-ml. centrifuge tube. Add 1 ml. of 2 per cent lead nitrate solution and mix. Add 1 drop of concentrated ammonium hydroxide and mix. Add 2 ml. of water, mix, and centrifuge until the upper layer is clear. Use 5 ml. of that solution for determination of calcium as the oleate and another aliquot for magnesium by Titan yellow.

Water and Other Solutions. Take a sample varying from 1-50 ml., according to the calcium content. If carbonate hardness is high, acidify with a drop of concentrated hydrochloric acid, hoil, cool, and hring hack to neutral with 1:10 ammonium hydroxide.

Tissue. Ash 1 gram of sample below redness in an electric oven. Take up with 1 ml. of 1:3 hydrochloric acid. Dilute with distilled water to 15 ml., warm, and make faintly alkaline to methyl orange on a spot plate by addition of a 10 per cent solution of ammcnium acetate. Filter off iron, if present, collecting the filtrate in a 25-ml. volumetric flask. Wash the precipitate and dilute to 25 ml. To 10 ml. of the above solution add 10 ml. of 95 per cent ethanol, and mix.

Blood. Transfer 2 ml. of whole blood to a beaker containing 10 ml. of concentrated nitric acid. Heat just below boiling for 2-3 hours. Increase the heat and evaporate to ahout 0.5 ml., avoiding spattering. If charring occurs add an additional 10 ml. of concentrated nitric acid and repeat the evaporation. Wash down the sides of the beaker and add 1 drop of 1 per cent phenolphthalein solution. Add 1:3 ammonium hydroxide until slightly alkaline. Heat to boiling for 2-3 minutes to remove excess ammonia and dilute to volume for use of aliquots.

Alternatively, add 8 ml. of 10 per cent trichloroacetic acid to a 2-ml. aliquot, mix well, and filter. Use a 5-ml. aliquot of the filtrate and determine calcium, preferahly by the reduction of phosphate.

Serum. Evaporate 5 ml. to dryness in a platinum dish and ash just helow redness. Extract the ash with 5 ml. of hot water and ash the residue. Dissolve the carhon-free ash in 2 ml. of 1:11 hydrochloric acid, comhine with the aqueous extract, and dilute to a suitahle volume for use of an aliquot. For precipitation as the oxalate use the serum directly as sample.[2]

[2] Julius Sendroy, Jr., *Proc. Soc. Exptl. Biol. Med.* 47, 136-8 (1941); *J. Biol. Chem.* 144, 243-58 (1942); *ibid.* 152, 539-56 (1944).

Urine. Transfer 2 ml. of sample to a tube and dilute to about 8 ml. Mix, add 0.5 gram of trichloroacetic acid, and shake. Dilute to 10 ml., mix, and filter. Collect exactly 5 ml. of filtrate, make distinctly alkaline with 1:1 ammonium hydroxide, and acidify with 30 per cent acetic acid until distinctly acid. Add sufficient yellow color to the standard, such as diphenylamine orange, to give the same color as shown by the sample.

Urinary Calculi. Weigh out 1.5 mg. of sample into a centrifuge tube calibrated at 10 ml. and use as the washed precipitate to determine by the ferric chloride and sulfosalicylic acid method (page 595). Prepare the standards as there shown and add the precipitating reagent to this sample in the same way as to the standards. If solution of the sample in the reagent is not complete, centrifuge before reading the color.

Feces. Weigh 5 grams into a silica dish and moisten with 2 ml. of concentrated sulfuric acid. Heat gently at first and finally more strongly until a white ash remains. Transfer the ash with 50 ml. of 1:2 hydrochloric acid to a 100-ml. volumetric flask. Dilute to volume, shake, and filter for the use of an aliquot.

Plant Tissue. The sulfur as sulfate, calcium, magnesium, sodium, and potassium are isolated as solution C in determination of lead (page 31). Heavy metals have been removed by dithizone. Take an aliquot for determination of calcium. Alternatively, use an aliquot of the solution prepared for determination of potassium (page 550).

Separation of Calcium from Magnesium. The technic described will isolate calcium from magnesium. Iron, aluminum, and strontium must be absent. Take a sample to contain 0.1-1.0 mg. and, if the volume exceeds 10 ml., concentrate to that level. Add a drop of methyl orange indicator, then 1:1 hydrochloric acid to an acid reaction, then 0.5 ml. in excess. Heat to boiling and add 1 ml. of saturated ammonium oxalate solution. Add 1:1 ammonium hydroxide until the reaction of the solution is alkaline. Let the solution stand for 2 hours to cool, stirring occasionally. Separate the precipitate by centrifuging or filtering, and wash with cold 0.1 per cent ammonium oxalate solution.

Dissolve the precipitate in 0.5 ml. of hot 1:1 hydrochloric acid, wash the filter thoroughly with hot water, and evaporate the solution to about 0.5 ml. Add 0.5 ml. of 30 per cent hydrogen peroxide to destroy oxalate and digest on a steam bath for about 30 minutes. Take up in water and

use as the sample solution, or dilute to a known volume for the use of aliquots.

Standard

Dry 5 grams of calcium carbonate at 110° for 3 hours. Weigh 2.4972 grams of the anhydrous salt, dissolve in 1:50 hydrochloric acid, make up to about 700 ml., and boil to expel carbon dioxide. Cool, neutralize with 1:5 ammonium hydroxide, and dilute to 1 liter with freshly boiled water. Each ml. contains 1 mg. of calcium.

Calcium as Oxalate

Precipitation of calcium with a minimum excess of oxalate yields a coarse, easily washed, slowly soluble precipitate of definite composition at a pH of 3.7 or less.[3] Rapid precipitation hastens the coprecipitation of any magnesium that may be present in solution. Decreasing the digestion period helps to eliminate this but may at the same time yield an incomplete and nonfilterable precipitate. Slow precipitation, a brief hot digestion in strong acid solution, adjustment of the cooled solution to a pH of 3.0,[4] and digestion at 25° for 30 minutes seem to yield best results.

A low pH prevents interference from moderate amounts of iron or aluminum. The precipitate in acid solution contains a little excess coprecipitated oxalic acid, whereas in alkaline solution there is a slight deficiency of oxalate. If sodium ion is high there may be coprecipitation of sodium oxalate. High sulfate content can cause coprecipitation of calcium sulfate with low oxalate content. Phosphate in an acid medium does not interfere. The minimum allowable pH varies slightly with the accuracy required, the amount of calcium present, the quantity of interfering substances, quantity of excess oxalate, and the volume. A formate buffer may be used to maintain the pH. Samples taken for analysis must be free from high concentrations of oxidizing or reducing substances, from metals forming insoluble oxalates, and from anions forming insoluble calcium salts. Varied methods have been used to determine the calcium oxalate.

The calcium content in the oxalate precipitate may be approximated

[3] Martha L. Washburn and M. J. Shear, *J. Biol. Chem.* 99, 21-41 (1932); Wilbur H. McComas, Jr. and William Rieman, 3rd, *Ind. Eng. Chem., Anal. Ed.* 14, 929-31 (1942); G. T. Pyne, *Analyst* 68, 330 (1943).

[4] Albert E. Sobel and I. Allan Kaye, *Ind. Eng. Chem., Anal. Ed.* 12, 118-20 (1940).

in water turbidimetrically[5] by adding to a 10-ml. aliquot 1 ml. each of 50 per cent acetic acid and of 20 per cent potassium oxalate solution, mixing after each addition. This may be compared after 10 minutes with a simultaneously prepared series of standards. No method is given for this technic.

The nephelometric estimation of calcium, although it requires but 10 minutes, is unsatisfactory due to the large size of the individual particles. The results are influenced by the stabilizer used, the presence of ammonium salts and excess precipitants, and the order of mixing the reagents. Accuracy ranges about 20 per cent.

A constant amount of ceric sulfate may be reduced by the oxalate and the excess determined by addition of potassium iodide in acid solution. This is read with an accuracy of ±2 per cent. As little as 0.002 mg. of calcium may be estimated.[6] With less, the intensity of the blue color formed with a 2 per cent starch solution may be read using a 600-mμ filter.

In use of the oxalate for reduction of potassium permanganate[7] and determination by difference, transmittance minima occur at 526 and 546 mμ. For photoelectric work with a yellow-green filter, transmittance around 530 mμ is suitable. Beer's law applies to such solutions containing 2.0-20.0 mg. of manganese per liter.

Calcium oxalate may be dissolved in a mixture of ferric chloride and hydrochloric acid and treated with sulfosalicylic acid. The color, which is stable, exhibits an intensity inversely proportional to the amount of calcium present. If phosphorus is present, a correction must be applied as it has 5 per cent of the inhibiting effect of oxalic acid on the color reaction. Calcium as oxalate may also be evaluated by the decolorization of ferric thiocyanate solutions. Magnesium does not interfere in the presence of ammonium ion but phosphates must be absent. Very small amounts of calcium, as in water or blood, may be estimated by this method. Results are accurate to ±2 per cent.

Procedure. *With Ceric Sulfate and Potassium Iodide.*[8] Transfer to a 15-ml. conical centrifuge tube a suitable volume of sample, such as

[5] W. D. Collins and Margaret D. Foster, *Ind. Eng. Chem.* 15, 1078-80 (1923).

[6] Julius Sendroy, Jr., *Proc. Soc. Exptl. Biol. Med.* 47, 130-8 (1941); *J. Biol. Chem.* 144, 243-58 (1942).

[7] Robert E. Scott and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* 17, 504-6 (1945); *Chemist-Analyst* 34, 81-6 (1945); cf. J. A. de Loureiro and G. J. Janz, *Biochem. J.* 38, 16-19 (1944); Judith E. Elliott and P. B. Pearson, *J. Lab. Clin. Med.* 31, 1262-6 (1946).

[8] Julius Sendroy, Jr., *J. Biol. Chem.* 144, 243-8 (1942).

serum, to contain 0.02-0.2 mg. of calcium and concentrate or dilute to 5 ml. If a photometer is not to be used take calcium standards diluted to the same volume. To each add sufficient 1:1 hydrochloric acid to neutralize the alkalinity, if any, and 2 drops in excess. Add 1 ml. of saturated ammonium oxalate solution to each tube, agitating after each addition. Add a drop of methyl orange indicator and 1:2 ammonium hydroxide, dropwise, until alkaline to that indicator. Cover with a rubber cap and let stand overnight.

Centrifuge for 5 minutes at 2600 rpm. Slowly decant or siphon off all but 0.2 ml. of the supernatant liquid. Wash the tube carefully with 3 ml. of 1:50 ammonium hydroxide. Tap to mix the ammonium hydroxide with the residual solution until upward movement of the precipitate just begins. Centrifuge and remove the supernatant liquid as before. Wash with 1 ml. of a mixture of equal parts of 95 per cent ethanol, ethyl ether and water,[9] mixing well with the precipitate and supernatant liquid. Allow the precipitate to settle and add 3 ml. of the same washing mixture, stirring the addition with the supernatant liquid only. Centrifuge for 5 minutes, withdraw the supernatant fluid, and repeat the washing with the alcohol-ether-water mixture. Dry the tubes for 45 minutes in an oven at 100-110° at a 15° angle.

Add 1 ml. of sulfuric acid, prepared by dilution of 2.7 ml. of concentrated acid to 100 ml., to each tube. Heat in a water bath, held at 90°, for 5 minutes. To the cooled tubes add 1 ml. of ceric acid sulfate prepared by dissolving 0.58 gram of ceric acid sulfate, $Ce(HSO_4)_4$, in 1:70 sulfuric acid to make 500 ml. of solution. Mix the fluids in the tube well. Allow to stand at room temperature for 30 minutes or in a water bath at 70° for 10 minutes. If the colorimetric reading is to be made after dilution to a known volume, transfer. Use four 1-ml. portions of water to wash the walls of the tube and effect quantitative transfer. Add 0.6 ml. of 1 per cent potassium iodide solution with a minimum of agitation. After 1 minute, make up to volume with a mixture of 40 parts of ethanol and 60 parts of water. Mix carefully. Set the photometer to 100 with a reagent blank. Adjust the temperature to 25° and read the yellow iodine color within an hour using a 400-mμ filter. Compare with a calibration curve.

With Ceric Sulfate, Potassium Iodide and Starch.[10] Follow the procedure for the determination of calcium oxalate by means of ceric sulfate

[9] L. Velluz and R. Deschaseaux, *Bull. soc. chim. biol.* 13, 797-808 (1931); Chi Che Wang, *J. Biol. Chem.* 111, 443-53 (1935).

[10] Julius Sendroy, Jr., and Alf S. Alving, *J. Biol. Chem.* 142, 159-70 (1942).

and potassium iodide through "Add 0.6 ml. of 1 per cent potassium iodide solution . . ." Add 0.5 ml. of 2 per cent starch solution and dilute sample and standard to a suitable volume. Adjust the temperature to 25°, mixing gently by rotation. Adjust the volume and read the blue color at 25° and 600 mμ. Compare against a calibration curve. This will determine smaller amounts of calcium but is less accurate.

With Potassium Permanganate. Run an aliquot of the sample containing about 2 mg. of calcium into a beaker and add 1 ml. of an acetate buffer for pH 4.7 containing 68.1 grams of sodium acetate trihydrate and 31.3 ml. of glacial acetic acid. Add 2 ml. of a reagent containing 5.738 grams of oxalic acid or 6.099 grams of sodium oxalate per liter. The pH should be 4.7 or below. Digest, reducing the volume to 2-8 ml., and cool. Filter the supernatant layer through an inorganic filter into a 100-ml. volumetric flask and wash the precipitate with three 2-ml. portions of water. In this method the excess of oxalic acid is to be determined. Therefore a bell jar will be used to hold the filter and contain the receiver. Remove the filtrate, and add 10 ml. of 1.1 sulfuric acid and 2 ml. of a potassium permanganate solution containing 2.877 grams per liter, which is 1 mg. of manganese per ml. When the color reaction is complete, dilute to volume. Read the transmittance within 20 minutes at 520 or 550 mμ and compare with a curve prepared under similar conditions.

Alternatively,[11] determine the oxalate in the precipitate. For this dissolve the precipitate in 20 ml. of hot 1:3 sulfuric acid and wash the filter with 10 ml. of hot water. Use a 50-ml. volumetric flask as receiver and add 2 ml. of 0.09 *N* potassium permanganate solution. Cool, dilute to volume, and read the color of the residual permanganate.

With Ferric Chloride and Sulfosalicylic Acid. Transfer 2 ml. of sample containing 5-15 mg. of calcium to a centrifuge tube calibrated at 10 ml. and standard calcium solutions to similar tubes. To each add 5 ml. of a 1 per cent sodium chloride solution, 0.5 ml. of 10 per cent ammonium chloride solution, and 10 drops of saturated ammonium oxalate solution. Stopper the tubes, mix, and let stand for 10-24 hours. Centrifuge and decant the clear upper layer. Wash the precipitates with three 10-ml. portions of water.

Prepare a reagent containing 10 ml. of 1 per cent ferric chloride solution and 10 ml. of concentrated hydrochloric acid diluted to 500 ml.

[11] Robert E. Scott and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* 17, 504-6 (1945); *Chemist-Analyst* 34, 81-6 (1945).

Add 2 ml. of this to each washed precipitate, working in an artificially lighted room. Add 2 drops of 20 per cent potassium acid iodate and 1 ml. of 2 per cent sulfosalicylic acid, and dilute each to 10 ml. The red color to be compared develops at once.

With Ferric Thiocyanate. Prepare the well-washed precipitated calcium oxalate by one of the previous methods. Dissolve in 1:50 hydrochloric acid. Prepare a ferric thiocyanate solution by mixing 5 ml. of a 0.3 per cent ammonium thiocyanate solution with 5 ml. of a 0.3 per cent ferric chloride solution, add a few drops of 1:1 hydrochloric acid to clarify the liquid, and dilnte to 25 ml. Let stand for one-half hour before use.

Transfer a 5-ml. aliquot of the clear calcium solution to a small tube. Add exactly 2 ml. of the prepared ferric thiocyanate solution and dilute with 1:20 hydrochloric acid solution to 10 ml. Compare lengthwise with a series of standard calcium oxalate solutions treated in the same manner. The color will vary inversely with the calcium content.

Calcium as the Phosphate

Calcium is determined indirectly by precipitation as tricalcium phosphate,[12] and subsequent reduction to molybdenum blue with one of several reagents. The blue color follows Beer's law.[13] Since the results of the molybdenum blue determination are a function of at least seven variables, the method is subject to a number of errors.[14] Because of these variables, the composition of the precipitate of calcium phosphate is not constant. The optimum pH ranges from 7-12. Arsenic must be absent and ferric ion must be reduced, as by a preliminary treatment with aluminum foil to inhibit its oxidizing action.[15]

Reduction of phosphomolybdate has been commonly effected by stannous chloride, 1,2,4-aminonaphthol sulfonic acid, or hydroquinone.

Procedure. *Reduction with Stannous Chloride.* To an aliquot of sample containing 0.1-1.0 mg. of calcium in a centrifuge tube add 1 ml. of 25 per cent sodium hydroxide solution, mix, and let stand for 5 minutes. If a magnesium precipitate appears, filter or centrifuge to separate

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[12] Rubens Salomé Pereira, *Rev. brasil. biol.* **4**, 263-70 (1944); *Rev. faculdade med. vet., Univ. Sao Paulo* **3**, 75-82 (1945).

[13] A. P. Briggs, *J. Biol. Chem.* **59**, 255-64 (1924); Carl Urbach, *Biochem. Z.* **241**, 226-7 (1931).

[14] Robert E. Scott and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* **17**, 504-6 (1945).

[15] Rubens Salomé Pereira, *Bull. soc. chim. biol.* **21**, 827-35 (1939).

it. Prepare an alkaline sodium phosphate mixture by dissolving 1 gram of trisodium phosphate in 50 ml. of water and mixing with 50 ml. of a 20 per cent solution of sodium hydroxide. If a precipitate forms allow it to settle for 24 hours, or centrifuge after 1 hour.

Add 5 ml. of this reagent to the sample, mix well, and set aside for an hour. Centrifuge, decant, and drain well, wiping off the edge of the tube. Prepare an alcoholic wash solution by mixing 58 ml. of 95 per cent ethanol and 10 ml. of amyl alcohol, and diluting to 100 ml. with water. Add 5 per cent sodium hydroxide solution dropwise until neutral to phenolphthalein.

To the precipitate of phosphate in the centrifuge tube, add 5 ml. of the wash solution and mix well with a glass rod. Centrifuge and decant. To the sample and two standards, add 1 ml. of 1.875 per cent sodium molybdate solution in 1:14 sulfuric acid. Add 10 ml. of water and mix. Prepare a stannous chloride solution by dissolving 10 grams of tin in 25 ml. of concentrated hydrochloric acid and diluting 0.5 ml. to make 100 ml. To the sample and standards add 5 ml. of this reagent, stopper with rubber, and invert immediately. After 1 minute compare colorimetrically with the standards prepared simultaneously, or read photometrically and compare with a standard curve.

Reduction with 1,2,4-Aminoaphthol Sulfonic Acid. Prepare a sample as for the stannous chloride method through "Centrifuge and decant." To the sample and standard, add 1 ml. of acid molybdate containing 6.25 per cent of ammonium molybdate in 1:4 sulfuric acid. The precipitate will completely dissolve. Add 10 ml. of water and mix. Prepare a 1,2,4-aminonaphthol sulfonic acid reagent by dissolving 30 grams of sodium bisulfite and 1 gram of sodium sulfite in 200 ml. of water. Add 0.5 gram of pure 1,2,4-aminonaphthol sulfonic acid, mix well, and filter. Keep in a dark bottle and renew every 2 weeks.

Add 5 ml. of the reagent and dilute to 15 ml. Mix well and let stand for 10 minutes in the dark. Read photometrically at 660 mμ and compare with a standardization curve. Alternatively balance against a similar standard prepared simultaneously.

Reduction with Hydroquinone. Transfer a sample containing 0.2 mg. of calcium to a 15-ml. test tube and add a drop of methyl red solution. Add 1:3 ammonium hydroxide with stirring until the color changes to yellow, then a few drops of 5 per cent acetic acid to produce a faint reddish color. Add 1 ml. of a 4 per cent solution of ammonium oxalate and rub the sides with a rubber-tipped rod until a precipitate forms.

Rinse off the rod and let the mixture stand 2 hours for complete precipitation. Centrifuge, wash the precipitate with 5 ml. of 0.5 per cent ammonium oxalate solution, and centrifuge again.

To the residue of calcium oxalate add 1 drop of concentrated hydrochloric acid and 0.5 ml. of 30 per cent hydrogen peroxide. Cover and heat for 30 minutes in a boiling water batb. Add 0.5 ml. of a 2 per cent solution of monopotassium phosphate and 3 drops of concentrated ammonium hydroxide. Let stand for 30 minutes to allow the calcium phosphate to precipitate and add 20 ml. of a solution containing 200 ml. of 95 per cent ethanol and 50 ml. of concentrated ammonium hydroxide per liter. This precipitates the calcium phosphate more completely, as it is less soluble in the alcoholic mixture. Centrifuge for 10 minutes and decant off the liquid. Add another 20 ml. portion of the above alcoholic mixture, stir, rubbing the sides of the tube with a policeman, again centrifuge, and decant off the liquid. To the residue of calcium phosphate add 5 ml. of water, 1 ml. of a 5 per cent ammonium molybdate solution, and 1 ml. of a reagent containing 30 grams of sodium bisulfita and 1 gram of hydroquinone in 200 ml. of phosphorous-free water. Dilute to 15 ml. and at the end of half an hour compara with 5 ml. of a standard monopotassium phosphate solution (page 659) similarly treated. Alternatively, read the transmittance and compare with a standard curve.

Calcium as a Fatty Acid Salt

Calcium may be estimated nephelometrically as an insoluble soap, sucb as the stearate,[16] oleate,[17] ricinoleate,[18] or laurate. Best results are obtained at concentrations of 0.04-2.0 mg. per 100 ml. Unless precautions are taken the magnesium compound will also be precipitated. Sometimes the calcium is precipitated and separated as the oxalate. The stearate reagent reqnires such a separation.

When potassium oleate is used in ammonical solution containing sodium lauryl sulfate, the degree of turbidity is proportional to the calcium content over the range 0.04-2.8 mg. of calcium per 100 ml. of solution.[19] The addition stabilizes the suspension over a long period of

[16] H. Lyman, *J. Biol. Chem.* **29**, 169-78 (1917); Michael Peech and Leah English, *Soil. Sci.* 57, 167-95 (1944).

[17] A. Grégoire, *J. Soc. Chem. Ind.* **42**, 437A (1923); A. Grégoire, E. Carpiaux, E. Larose and T. Sola, *Bull. soc. chim. Belg.* 32, 123-30 (1923); Abraham Saifer and Franklin D. Clark, *Ind. Eng. Chem., Anal. Ed.* 17, 757-9 (1945).

[18] H. Lyman, *J. Biol. Chem.* **21**, 551 (1915); F. K. Lindsay and R. G. Bielenberg, *Ind. Eng. Chem., Anal. Ed.* **12**, 460-3 (1940).

[19] Abraham Saifer and Franklin D. Clark, *Ind. Eng. Chem., Anal. Ed.* **17**, 757-9 (1945).

time and prevents precipitation of magnesium. The method was developed primarily for water analysis. Addition of ammonia removes interfering ions and also helps to stabilize the colloidal suspension. The ammonium hydroxide concentration should be kept below 0.5 per cent. Over the range 8-350 ppm. of calcium as carbonate, it may be determined with an average error of ±4 per cent. The optimum transmittance occurs at 420 mμ. Magnesium does not produce turbidity with the ricinoleate in the presence of ammonium ion. The method may be modified to include both calcium and magnesium.

Procedure. *As the Stearate.*[20] A modified reagent gives greater uniformity of dispersion. Dissolve 4 grams of pure stearic acid and 0.5 gram of pure oleic acid in 425 ml. of 95 per cent ethanol by warming on the water bath. Add to the warm solution 20 grams of ammonium oxalate in 100 ml. of warm water. Cool and add 425 ml. of 95 per cent ethanol, 50 ml. of water, and 20 ml. of concentrated ammonium hydroxide. Filter and preserve the reagent from evaporation.

Precipitate the calcium oxalate as usual and filter. Ignite the paper and precipitate until decomposition is complete. Take up the residue of calcium oxide in 0.5 ml. of 1:1 nitric acid and evaporate just to dryness. Dissolve in water and dilute to 50 ml. in a volumetric flask. Take an aliquot to contain 0.02-0.6 mg. of calcium and transfer to a 50-ml. flask. Add 10 ml. of water and dilute to 50 ml. with the reagent. Warm at 30-40° for 15 minutes and read the obscuring power instrumentally. Compare with curves prepared from standard solutions under similar conditions.

As the Oleate. Prepare a potassium oleate solution[21] by shaking 7.05 grams of oleic acid with a solution of 1.60 grams of potassium hydroxide in 5 ml. of distilled water. Transfer to a flask using 50 ml. of 70 per cent ethanol. Reflux for an hour and dilute to 250 ml. To 100 ml. of 3 per cent Duponol PC.[22] solution add 20 ml. of the potassium oleate reagent. Allow to stand for 12 hours and filter or centrifuge to remove any precipitate present. The reagent is stable at room temperature but will cloud out at lower temperatures. It can be redissolved at 37°.

If the sample is water, use 45 ml., otherwise use an appropriate

[20] N. M. Miloslavskii and E. G. Vavilova, *Zavodskaya Lab.* 6, 28-33 (1937).

[21] A. Romeo and V. Gambardella, *Chim. ind. agr. biol.* 17, 471-6 (1941).

[22] E. I. du Pont de Nemours and Co., Inc., Dyestuffs Department, Wilmington, Del.

aliquot in a 50-ml. volumetric flask. Neutralize with concentrated ammonium hydroxide until just alkaline to litmus and add 1 ml. in excess. Mix, dilute volumetrically to 50 ml., and mix. Allow to stand for 15 minutes and filter any precipitate that forms. Pipet 2-ml. and 5-ml. aliquots into colorimeter tubes. Dilute the first to 5 ml. Take a third tube as blank and in it dilute 0.5 ml. of 1:10 ammonium hydroxide to volume.

To each tube add 5 ml. of reagent, mix, and place in a bath at 20° for 30 minutes. Use the blank to set the transmittance of the instrument at 100 per cent. Read the sample which can be read most accurately and compare with a calibration curve. By incorporation of any correction in such a curve, further correction need not be applied to the results.

By Sulforicinoleate Reagent. To a 12.0-ml. aliquot of sample in a 15-ml. centrifuge tube add, with agitation after each addition, 1 ml. of a 2 per cent lead nitrate solution, 1 drop of concentrated ammonium hydroxide, and 2 ml. of water. Centrifuge until the supernatant liquid is clear. Transfer the upper layer to a 25-ml. flask and wash the precipitate once with 1:10 ammonium hydroxide.

Prepare a reagent solution by dissolving 80 grams of sodium sulforicinoleate in 896 ml. of 0.4 per cent sodium hydroxide solution and diluting to 1 liter. Add 4 ml. of this reagent and 2 ml. of a 1 per cent solution of oleic acid in 95 per cent ethanol. Fill to the mark with water and after 5 minutes read the turbidity. Compare with standards prepared at the same time or read the transmittance and compare with a curve.

As the Laurate.[23] Weigh out 8 grams of lauric acid and heat with 888 ml. of water until melted. Add 4.5 ml. of 16.8 per cent potassium hydroxide and agitate vigorously until the potassium laurate is fully dissolved. Store the solution in a Pyrex bottle while still hot, thus avoiding absorption of carbon dioxide. To 200 ml. of sample solution containing 0.1-0.5 mg. of calcium, add 10 ml. of 16.8 per cent potassium hydroxide solution and 10 ml. of reagent. Mix well and read after 20 minutes. Subtract blanks on the sample as received and on 200 ml. of water treated as described for the sample.

Miscellaneous

Calcium is precipitated from solutions free from protein as a calcium-

[23] F. H. Long, *Bulletin of Wilkins-Anderson Co.*, December, 1946.

hydroxyquinoline complex by addition of 8-hydroxyquinoline.[24] By treatment with ammonium chloride solution the calcium complex is dissolved and the corresponding magnesium precipitate is not. The calcium is estimated indirectly by the use of Folin's reagent. Results were accurate to 2 per cent. A high magnesium content does not lower the accuracy of the method.

Unsatisfactory results are obtained in the corresponding magnesium determination if the hydroxyquinoline reagent is not freshly prepared and all reagents are not negative to the starch-iodide test.[25]

To a suitable aliquot of sample and a corresponding standard, add 0.15 ml. of a solution of 50 grams of sodium potassium tartrate in 100 ml. of distilled water. Add 0.25 ml. of 4 per cent sodium hydroxide solution and mix. Add 0.15 ml. of a 5 per cent alcoholic 8-hydroxyquinoline solution dropwise. Mix and after 10 minutes stir vigorously with a glass rod. After 2 minutes, when the maximum turbidity has been reached, place in a boiling water bath for 2 minutes. Cool to room temperature and let stand for 2 minutes to complete the calcium precipitation. Centrifuge, decant, and wash 4 times with 1 ml. portions of the alkaline tartrate solution and 1 drop of 4 per cent sodium hydroxide solution.

Decant and add 1 ml. of a solution containing 34 ml. of concentrated ammonium hydroxide in 1 liter of 5 per cent ammonium chloride solution. Mix well and place the tube in a water bath at 80°. Gradually raise the temperature to boiling and maintain for 1 minute. Add 1 drop of concentrated ammonium hydroxide, and mix. After 3 minutes add another drop of concentrated ammonium hydroxide and mix. Filter while hot through an inorganic filter into a 10-ml. volumetric flask. Wash the precipitate 3 times with 0.5-ml. portions of distilled water. Add 0.5 ml. of 0.01 *N* hydrochloric acid to the solution and washings. In another flask take 2 ml. of standard calcium solution containing 0.01 mg. of calcium per ml. Add 1 drop of concentrated ammonium hydroxide and a few ml. of distilled water.

To each flask add 1.2 ml. of 20 per cent sodium carbonate solution and mix. Add 1 ml. of phenol reagent (page 623) to the sample and standard and mix. Place the flasks in a boiling water bath immersed to half the depth of the solution and heat for 5 minutes. Dilute to the mark and mix. Let cool, again dilute to the mark, and mix. Compare or read photometrically.

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[24] Shun ichi Yoshimatsu, *Tôhoku J. Exptl. Med.* **14**, 29 (1929); *ibid.* **15**, 355 62 (1930); R. Berg, W. Wolker and E. Skopp, *Mikrochem. Emich Festschr.* 1930, 18-22.

[25] F. Eichholtz and R. Berg, *Biochem. Z.* **225**, 352-7 (1930).

aliquot in a 50-ml. volumetric flask. Neutralize with concentrated ammonium hydroxide until just alkaline to litmus and add 1 ml. in excess. Mix, dilute volumetrically to 50 ml., and mix. Allow to stand for 15 minutes and filter any precipitate that forms. Pipet 2-ml. and 5-ml. aliquots into colorimeter tubes. Dilute the first to 5 ml. Take a third tube as blank and in it dilute 0.5 ml. of 1:10 ammonium hydroxide to volume.

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[23] F. H. Long, *Bulletin of Wilkins-Anderson Co.*, December, 1946.

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Decant and add 1 ml. of a solution containing 34 ml. of concentrated ammonium hydroxide in 1 liter of 5 per cent ammonium chloride solution. Mix well and place the tube in a water bath at 80°. Gradually raise the temperature to boiling and maintain for 1 minute. Add 1 drop of concentrated ammonium hydroxide, and mix. After 3 minutes add another drop of concentrated ammonium hydroxide and mix. Filter while hot through an inorganic filter into a 10-ml. volumetric flask. Wash the precipitate 3 times with 0.5-ml. portions of distilled water. Add 0.5 ml. of 0.01 N hydrochloric acid to the solution and washings. In another flask take 2 ml. of standard calcium solution containing 0.01 mg. of calcium per ml. Add 1 drop of concentrated ammonium hydroxide and a few ml. of distilled water.

To each flask add 1.2 ml. of 20 per cent sodium carbonate solution and mix. Add 1 ml. of phenol reagent (page 623) to the sample and standard and mix. Place the flasks in a boiling water bath immersed to half the depth of the solution and heat for 5 minutes. Dilute to the mark and mix. Let cool, again dilute to the mark, and mix. Compare or read photometrically.

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[24] Shun ichi Yoshimatsu, *Tôhoku J. Exptl. Med.* 14, 29 (1929); *ibid.* 15, 355-62 (1930); R. Berg, W. Wolker and E. Skopp, *Mikrochem. Emich Festschr.* 1930, 18-22.

[25] F. Eichholtz and R. Berg, *Biochem. Z.* 225, 352-7 (1930).

Picrolonic acid precipitates calcium in neutral solution and, when the solution of the precipitate is made alkaline, an orange to red color appears which is proportional to the concentration of picrolonic acid.[26] The color is stable in the dark for 48 hours. Magnesium, potassium, sodium, and ammonium ions do not interfere. Iron and aluminum should be separated by precipitation, or kept in solution in the final alkaline medium by the addition of thiosalicylic acid.

For application of this method, take an aliquot of sample containing 0.1-2.5 mg. of calcium. Neutralize and dilute or concentrate to about 9 ml. Transfer to a container calibrated at 10 ml., add 0.25 ml. of 10 per cent alcoholic thiosalicylic acid solution to prevent interference by iron or aluminum, and dilute to volume. Heavy metals must be absent. Pipet a 2-ml. aliquot into a centrifuge tube containing 6 ml. of 0.25 per cent solution of picrolonic acid. Mix and chill in ice water for 4 hours. Centrifuge and decant. Wash the precipitate with 3 successive portions of anhydrous ether. Evaporate any residual ether. Take up the residue in hot water. Add 1 ml. of saturated bromine water to this solution and heat on a steam bath for 10 minutes. Add 10 ml. of ethanol to remove excess bromine and let cool. Add 8 per cent sodium hydroxide solution until a maximum reddish yellow color is developed. Compare with a standard or read the transmittance.

Chloroanilic acid, 2,5-dichloro-3,6-dihydroxyquinone, is intensely colored and forms a very insoluble calcium salt. The reduction in color may be used for colorimetric determination of calcium.[27] The minimum transmittance is around 550 mμ. Transmittance values conform to Beer's law up to 400 ppm. at 550 mμ and over the range 80-280 ppm. at 430 mμ. Hydrogen-ion concentration must be closely controlled. The maximum diminution of color is obtained in 3 hours. The color transmittance drifts somewhat with the age of the stock solution, regardless of light exposure. Ferric ions form a characteristically colored interfering complex. An aluminum complex formed does not alter the color. Copper, manganese, barium, and strontium precipitate the reagent. Sodium, potassium, and magnesium may be coprecipitated with calcium and give high results for calcium.

Transfer a sample containing no more than 0.01 gram of calcium to a 500-ml. volumetric flask. It must be acid with no more than 1 ml. of N acetic acid. Dilute to about 100 ml. and add 100 ml. of 0.15 per

[26] F. Alten, H. Weiland and E. Knippenberg, *ibid.* 265, 85-9 (1933); Gunther Cohn and I. M. Kolthoff, *J. Biol. Chem.* 147, 705-19 (1943).

[27] A. Barreto, *Rev. quím. ind.* (Rio de Janeiro) 14, No. 163, 18 (1945); Edward H. Tyner, *Anal. Chem.* 20, 76-80 (1948).

cent solution of chloroanilic acid. Mix well, let stand for an hour, and dilute to volume. Filter and balance against a 0.03 per cent solution of chloroanilic acid, calculating the loss in color in terms of predetermined calcium standards, or read the transmittance at 550 mμ.

Alizarin in ethanol solution is used in the absence of magnesium to determine as little as 0.1 mg. of calcium with an accuracy of $\pm$2-5 per cent.[28] Calcium is quantitatively precipitated with nickel nitrite as the complex $K_2CaNi(NO_2)_6$.[29] The nitrite content is then estimated by means of an antipyrine reagent.[30] The reaction is sensitive to 0.1 mg. of nitrite per liter of water. Copper, manganese, aluminum, iron, zinc, and magnesium do not precipitate. The method is accurate to within 4 per cent.

Calcium and magnesium in blood or water are determined by measuring the green turbidity produced by ammonium ferrocyanide in a 1:1 alcoholic solution.[31] Barium and strontium react only in concentrated solutions of their salts. About 1 ppm. can be detected, and the accuracy is to 3 per cent.

Sodium tungstate precipitates calcium as well as barium, strontium, aluminum, zinc, lead, mercury, and copper. The tungsten may be colorimetrically estimated by reduction with titanous chloride as a measure of the calcium present[32] This [33] is applicable in the presence of 500 times the magnesium equivalent. Add sodium carbonate until neutral but without precipitating calcium carbonate. Then add 2.5 ml. of 0.5 per cent sodium tungstate solution. Evaporate nearly to dryness and dilute to 25 ml. Centrifuge and remove a 5-ml. aliquot of the upper layer. To this add 1 ml. of 0.1 *N* hydrochloric acid and 5 ml. of titanous chloride solution equivalent to 2 mg. of ferric ion. Compare with tungstate standards (page 465).

The hardness of water is determined by the effect of calcium and magnesium on tropeolin 00.[34] Therefore this is a method of determination of calcium only if magnesium is absent. The reagent is also a pH

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[28] P. P. Laidlaw and W. W. Payne, *Biochem. J.* 16, 494-8 (1922).

[29] M. Mousseron, *Bull. soc. chim. biol.* 12, 1014-21 (1930).

[30] A. Astruc and M. Mousseron, *Compt. rend.* 190, 1558-9 (1930); M. Mousseron, *Bull. soc. chim. biol.* 13, 831-4 (1931).

[31] F. Feigl and F. Pavelka, *Mikrochemie* 2, 285-91 (1924).

[32] M. Mousseron and N. Bouisson, *Bull. soc. chim. biol.* 12, 482-90 (1930).

[33] Émile Rinck and Hélène Ostertag, *Compt. rend.* 224, 1108-10 (1947).

[34] G. Kuhl, Belgian Patent 445,440 (1942); S. M. Drachev, *Zavodskaya Lab.* 11, No. 1, 46-8 (1945); V. I. Adamovich, *Hig. i Sanit.* (U.S.S.R.) 10 No. 12, 16-19 (1945)

indicator in changing from red to yellow in the range pH 1.3-3.2. For the determination the yellow form is used.

Prepare a tropeolin reagent by dissolving 0.4 gram of tropeolin 00 in 50 ml. of ethanol and dilute to 100 ml. with water. Prepare a series of standards from artificial hard water, or from naturally hard water which has been analyzed. The total calcium and magnesium, expressed as calcium carbonate, should not exceed 400 ppm. Place 1 ml. of sample and of the standards in uniform tubes. To each add 0.5 ml. of tropeolin reagent and mix. If the sample requires standards higher than 200 ppm. of hardness increase the amount of reagent to 1 ml. Mix and compare after 2 minutes.

An indirect method[35] for estimation of calcium consists of precipitation of calcium molybdate and thereafter estimation of the combined molybdenum as the red-orange complex obtained with thiocyanate (page 478).

[35] Nicolô Gandolfo, *Gass. chim. ital.* 75, 62-70 (1945).

CHAPTER 42

BARIUM

ALTHOUGH not widely distributed, barium is important. Aside from its occurrence in minerals, it is physiologically significant as being highly poisonous. The colored compounds are strictly limited. So the methods for determination of barium are limited to colorimetric estimation as the chromate or turbidimetric estimation as the sulfate. It follows from the first of these that the barium chromate may be isolated, redissolved, and methods of determination of chromate applied as indirect measures of the barium.

SAMPLE

Any sample solution free from other metals which precipitate with the reagent is suitable. Mainly this means that if lead is present there must be ammonium acetate added in sufficient amount to keep it from precipitating.

STANDARD

Dissolve 1.7787 grams of barium chloride dihydrate, $BaCl_2 \cdot 2H_2O$, in water and dilute to 1 liter. This is equivalent to 1 mg. of barium per ml.

BARIUM AS THE CHROMATE

When ammonium chromate is added to barium ions, a yellow precipitate of barium chromate forms which, when dissolved in hydrochloric acid, affords a means of estimating barium colorimetrically.[1] Complete precipitation requires careful control of the pH of the solution. The maximum color sensitivity of chromate solutions lies between 0.004 and 0.008 *N*.[2] Best results are obtained in solutions containing 0.3-1 mg. of barium per ml. If the solution is more concentrated, measurements must be made through shallower solutions, and this introduces additional error. Less than 0.2 mg. per ml. produces an insufficiently colored solution. The use of filters extends these limits. For solutions containing 0.01-1 mg. per ml. a blue filter yields greatest accuracy. Up to

[1] H. A. Frediani and B. J. Babler, *Ind. Eng. Chem., Anal. Ed.* 11, 487-9 (1939).
[2] David W. Horn, *Am. Chem. J.* 35, 253-8 (1906).

1.5 mg. per ml., white light may be used. For more concentrated solutions containing up to 6 mg. of barium per ml. a green filter should be used. If strontium is present it will interfere. Sodium, potassium, calcium, and magnesium do not interfere.

Barium is also determined indirectly by precipitating with a known excess of chromate, filtering, and measuring the color of the excess chromate.[3]

Procedure. Neutralize an aliquot of sample solution with 20 per cent sodium hydroxide solution. Add 3 drops of glacial acetic acid and 10 ml. of 30 per cent ammonium acetate solution. Heat to boiling, and add dropwise 10 per cent ammonium chromate solution until no more precipitate forms when the reagent is added. Add 10 drops in excess. Digest for 30 minutes and decant through a sintered glass crucible. Wash the precipitate thoroughly with hot water. Dissolve the precipitate in 10 ml. of 1:1 hydrochloric acid, and dilute to 100 ml. Compare with similarly prepared standards or read the transmittance with a blue filter.

Barium as the Sulfate

Sulfate radical is customarily estimated turbidimetrically by addition of barium chloride. The reverse use of the method requires addition of 1 ml. of 1:10 sulfuric acid to the barium solution instead of barium chloride solution to a sulfate solution. For further details see the turbidimetric method for determination of sulfate (page 767).

Miscellaneous

When 1 drop of 10 per cent tannic acid solution is added to 10 ml. of a dilute, slightly alkaline solution of a barium, calcium, or strontium salt, a yellow-blue color develops [4] which fades to yellow in 3-5 minutes. If the solution is too concentrated a blue-green precipitate forms, making dilution necessary. If less than 0.1 mg. per liter is present only the yellow color appears. Magnesium in concentrations over 30 per cent interfere. This color lasts about three minutes, and therefore is not very satisfactory for colorimetry.

Sodium rhodizonate and gelatin produce a red color in slightly acid barium solutions.[5] The color is unstable in the light.

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[3] Stefan Christov, *Z. anal. Chem.* **125**, 278-86 (1943).

[4] G. Ammer and H. Schmitz, *Wasser* **8**, Pt. II, 161-8 (1934).

[5] A. Friedrich and S. Rapoport, *Mikrochemie* **14**, 41-8 (1933).

CHAPTER 43

CERIUM

CERIUM is one of the series of rare earths, of which it is the most abundant. Nevertheless it is rarely encountered, having as one of its main functions the activation of thorium oxide in incandescent mantles and, as another, the fast-fading use in the flaming arc light. Although many color reactions with organic reagents have been developed for the detection of cerium, few have been adapted to quantitative colorimetric determination. As in the case of other rare earths it is assumed that samples are available in aqueous solution. Since tetravalent cerium ion is yellow, that furnishes one direct means of determination.

STANDARD

Weigh out 1.228 grams of pure ceric oxide. Add 100 ml. of 1:2 sulfuric acid and heat until solution is complete. Dilute to 1 liter, of which each ml. contains 1 mg. of cerium as the sulfate.

CERIUM AS PHOSPHATE BY MOLYBDENUM BLUE

Use the method outlined for lanthanum (page 610) taking a sample containing about 50 mg. of cerium and a similar standard.[1]

CERIUM BY PERSULFATE

In the absence of chromium and manganese which would be oxidized to chromate and permanganate respectively, cerium is oxidized to the ceric form for determination. Chlorides, fluorides, and phosphates interfere.

Procedure. Take an aliquot of sample containing 0.5-5.0 mg. of cerium. Add 1 ml. of 1:1 sulfuric acid if not already present and, if chlorides are present, evaporate to fumes. Dilute to 10 ml. and add 0.5 ml. of 0.1 per cent silver nitrate solution and 0.2 gram of ammonium persulfate. Boil gently for 5 minutes and cool. Dilute to volume and

[1] Eugen Bamann and Emil Heumüller, *Ber.* 75B, 1514-17 (1942).

read the transmittance. Compare with a curve prepared from a standard cerium solution.

Cerium by Brucine

If a solution containing ceric ion is made slightly acid with sulfuric acid, and a solution of brucine acetate is added, an orange-red color is formed.[2] The intensity of the color depends upon how long the solution is kept after its preparation, the optimum length of time being 15-20 hours. The reaction follows Beer's law. Ammonium persulfate, if present, must be decomposed in a preliminary treatment. No other metal ion interferes. Other oxidizing agents give more or less the same result. The intensity of coloration is about 5 times that of ceric ion.

Procedure. Prepare the sample as for direct reading of ceric ion (page 607) through the step "Boil gently for 5 minutes . . ." Add more water if necessary and continue to boil for 10 minutes longer to insure decomposition of all persulfate. At the end of the boiling, cool and add 0.25 ml. of a 0.1 per cent solution of brucine in 1:35 sulfuric acid. Let the solution stand overnight and either compare with standards simultaneously prepared or read the transmittance with a blue filter.

Cerium by Pyrogallol or Gallic Acid

Pyrogallol[3] and gallic acid[4] in ammoniacal solution react with cerium ions to form colored solutions or precipitates.[5] In the case of pyrogallol, the color formed varies with the concentration of reagents, from a violet sol to a dark purple-red precipitate, and in the case of gallic acid from colorless to a light-violet sol which may change to yellow-brown or to green. For best results in terms of the sol form, the concentration of ammonium hydroxide should be increased and that of pyrogallol decreased. Sodium sulfite stabilizes the solution.

Although thorium, lanthanum, and titanium interfere, iron, chromium, aluminum, manganese, nickel, cobalt and uranyl ions do not.

[2] F. M. Shemyakin, V. A. Volkova and A. S. Bozhko, *J. Gen. Chem.* (U.S.S.R.) **8**, 452-5 (1938); F. M. Shemyakin and V. A. Volkova, *ibid.* **9**, 698-700 (1939).

[3] F. M. Shemyakin, *Z. anorg. allgem. Chem.* **217**, 272-6 (1934).

[4] F. M. Shemyakin, *Zavodskaya Lab.* **3**, 1090-1 (1934).

[5] F. M. Shemyakin and T. V. Vashedchenko, *J. Gen. Chem.* (U.S.S.R.) **5**, 667-74 (1935); F. M. Shemyakin, A. V. Veselova and M. I. Vladimirova, *Zavodskaya Lab.* **5**, 231-2 (1936).

Atmospheric oxidation may be prevented by using a nonaqueous protective layer.

Procedure. *Gallic Acid.* Mix 2.7 ml. of sample solution containing 0.03-0.07 mg. of cerium with 2.7 ml. of 0.02 per cent gallic acid solution. Add 2 ml. of ether, toluene or mineral oil to form a protective layer on the surface. Add 5.3 ml. of a 1 per cent solution of sodium sulfite containing 0.7 ml. of concentrated ammonium hydroxide per 100 ml. Mix carefully and dilute to a known volume with the protective layer above the mark. Compare with a series of standards similarly developed from a known cerium solution.

Pyrogallol. Substitute a similar solution of pyrogallol for the gallic acid.

Miscellaneous

A white precipitate forms when a dilute solution of cerium salt is treated with potassium carbonate, which dissolves in excess of potassium carbonate. This is oxidized by the atmosphere on standing to the perceric carbonate, $Ce_2(CO_3)_3O_3 \cdot 4K_2CO_3 \cdot 12H_2O$, whose intense yellow color is suitable for the colorimetric determination of cerium by measurement of the transmittance.[6]

[6] Jenö Plank, *Z. anal. Chem.* 116, 312-15 (1939).

CHAPTER 44

LANTHANUM

LANTHANUM is another of the rare earths, less frequently encountered than cerium. Since it has no major industrial applications, work on its determination by colorimetric means has been limited to the molybdenum blue method of indirect determination of the phosphate. The sample is assumed to be in aqueous solution, of sufficient acidity to maintain solubility.

STANDARD

Weigh out 1.173 grams of pure lanthanum sesquioxide and add 100 ml. of 1:2 sulfuric acid. Heat until solution is complete, cool, and dilute to 1 liter. Each ml. contains 1 mg. of lanthanum as the sulfate. Other salts are similarly prepared from the oxide.

LANTHANUM AS PHOSPHATE BY MOLYBDENUM BLUE

Because of the insolubility of many rare earth phosphates it is feasible to use this indirect method. Lanthanum is precipitated as orthophosphate in the presence of sodium acetate, and determined by conventional methods of estimation of phosphate as molybdenum blue.[1] Cerium and other rare earths interfere. For satisfactory results, enough precipitating agent is added so that the proportion of phosphate to lanthanum ions is 2:1. If the determination is made photoelectrically the average deviation from the theoretical value is ±1 per cent, and generally the error is under ±0.5 per cent.

Procedure. As aliquot use a quantity of sample in nitric acid solution comprising approximately 50 mg. of sample taken as lanthanum nitrate sesquihydrate. Add 3 ml. of 8 per cent sodium acetate solution which has been diluted with 17 ml. of water. Add dropwise with swirling 5 ml. of 0.4 per cent monosodium phosphate solution. Let the precipitate stand for 0.5-1 hour, with frequent shaking.

Centrifuge and decant. Wash the precipitate with 0.25 per cent

[1] Eugen Bamann and Emil Heumüller, *Ber.* 75B, 1514-17 (1942).

sodium acetate solution, stirring the precipitate thoroughly with just enough of the solution to obtain a smooth paste. Add an additional 40 ml. of the wash liquid, stir vigorously, and centrifuge. Decant and repeat the washing procedure twice more. Decant, dissolve the precipitate in a drop of 1:3 hydrochloric acid, and transfer to a 200-ml. volumetric flask. Determine lanthanum in an aliquot colorimetrically by reduction of the phosphate to molybdenum blue (page 668) using any of the conventional methods of development of the color. It may be read against a standard, or the transmittance may be read against a standard phosphate curve. The phosphate as P_2O_5 multiplied by 1.955 gives the amount of lanthanum as the element.

If the lanthanum concentration is very small, use a sample containing as little as 0.2 mg. of lanthanum nitrate or 0.06416 mg. of lanthanum and cut the volume of reagents to 0.20 ml. of 8 per cent sodium acetate, and 0.05 ml. of 0.5 per cent monosodium phosphate, and extend the length of time required for precipitation to 24 hours. Make the corresponding changes in wash solution and in development of color.

CHAPTER 45

MAGNESIUM

MAGNESIUM is a widely distribnted element in minerals, water, and biological samples. It follows that samples may be from almost any source in the mineral, animal, or vegetable kingdoms.

The determination of small amounts of magnesium is difficult because it forms no colored componnds directly. The familiar gravimetric precipitation of magnesium ammonium phosphate has led to its indirect determination by the amount of phosphate present. Usually this is even more indirect by conversion of the phosphate to phosphomolybdate and formation of molybdenum blue. The varied steps of manipulation offer plenty of opportunity for error to creep in. The precipitation of magnesium hydroxyquinolate in alkaline solution is the basis of another method, usually indirect as the ferric hydroxyquinolate. There is a recent trend toward use of the sorption complex formed by the hydroxide with Titan yellow. Various lakes of magnesium with other dyestuffs are additional methods not unlike that with Titan yellow.

SAMPLES

Aluminum Alloys.[1] Weigh out 0.6 gram of alloy containing 0.20-2.00 per cent of magnesium, or 1.2 grams of alloy if the magnesium content is below 0.2 per cent. Add 20 ml. of 1:1 hydrochloric acid and warm if necessary to start the reaction. Remove from the heat when the reaction has ceased and add 10 ml. of concentrated nitric acid. Heat to complete solution. If silicon is present add 48 per cent hydrofluoric acid in 1-2-ml. portions, warming after each addition, until it is completely dissolved. Evaporate to about 10 ml. to remove acids, but avoid heavy formations of difficultly soluble crystals. Dilute with 30 ml. of hot water, heat to boiling, and dissolve all salts as rapidly as possible. Add, with boiling after each addition, 20 ml. of 30 per cent sodium tartrate solution and 20 ml. of 30 per cent tartaric acid solution. Add 8-10 drops of 0.04 per cent bromocresol purple solution in methanol, heat to 90°, and, while at this temperature, add dropwise a solution of 20 per cent sodium

[1] Henry C. Deterding and Richard G. Taylor, *Ind. Eng. Chem., Anal. Ed.* 18, 127-9 (1946); cf. F. Sinigaglia, *Z. Ver. deut. Chem., Beih.* 48, 104-6 (1944).

hydroxide until a distinct purple color appears. Add 10 ml. of 30 per cent potassium cyanide solution, boil for 1 minute and, while keeping the solution at a vigorous boil, add 30 ml. of 20 per cent sodium hydroxide solution. When the solution is clear, add 100 ml. of hot water slowly and keep at boiling for 1-2 minutes. Remove from the heat, swirl slowly, and cool slightly. Magnesium hydroxide appears as fine particles. Digest on a hot plate at 90-95° for 3-5 minutes to coagulate the precipitate, and filter. Wash well and discard the filtrate and washings. The precipitate and paper will still retain some aluminum which must be removed.

Dissolve the washed magnesium hydroxide precipitate with 30 ml. of hot 1:4 acetic acid, catching the filtrate in the original flask. Wash the paper with hot water and combine with the filtrate, keeping the volume within 60-75 ml. Add 10 ml. of 10 per cent ammonium benzoate solution and heat just to boiling. Remove from the hot plate and allow to stand for 2 minutes. Filter and wash with warm water, keeping the volume at 100-125 ml. Discard the washed precipitate. If the magnesium content is low, add 2-3 ml. of 30 per cent tartaric acid to aid in subsequent precipitation of magnesium hydroxyquinolinate. Add 5-6 drops of a 0.04 per cent solution of bromocresol purple and neutralize the solution to a definite purple color by the dropwise addition of 1:5 ammonium hydroxide. Use as sample for the determination of magnesium, preferably as hydroxyquinolate in acid solution.

Alternatively,[2] treat a 0.5-gram sample with 15 ml. of 25 per cent sodium hydroxide solution. To the residue add 10 ml. of 1:3 sulfuric acid and evaporate to fumes. Dilute and filter, and neutralize the filtrate by the dropwise addition of a 10 per cent sodium carbonate solution. Heat to boiling, add excess of a creamy suspension of zinc oxide, and oxidize the hot solution with just enough 1.5 per cent potassium permanganate to form a pink color. Boil to discharge the color, filter into a 500-ml. volumetric flask, and dilute to volume for use of 25-ml. aliquots in the determination of magnesium by the Titan yellow method.

Zinc-aluminum-copper and Zinc-aluminum-iron Alloys. A solution was prepared for determination of copper which also contains the magnesium (page 81). Use a suitable aliquot of the solution and make definitely acid with concentrated nitric acid. Remove the copper by electrolytic deposition (page 82). Evaporate the solution to dryness and volatilize the ammonium salts. Add a few drops of concentrated

[2] F. Sinigaglia, *Alluminio* 11, 157-61 (1942); cf. E. Kreibich and H. Baümler, *Aluminium* 20, 528-31 (1938); Lothar Koniakovsky, *ibid.* 25, 208-13 (1943).

nitric acid and ignite again. Add 2 ml. of 1:2 sulfuric acid and ignite to copious sulfur trioxide fumes. Take up with water for nse as the sample.

Lead-magnesium Alloys.[3] Dissolve a 0.5-gram sample in 15 ml. of concentrated nitric acid hy heating gently on a sand hath. Cool and add 50 ml. of a 3 per cent sodium oxalate solntion. Shake vigorously, heat on a sand hath almost to hoiling and, with continuous agitation, add 10-15 ml. of cold water. Cool, transfer to a 200-ml. volumetric flask, and dilute to volume. Mix and filter. Discard the first portion of the filtrate and reserve the remaining filtrate for use of 25-ml. aliquots in the determination of magnesium hy the Titan yellow method.

Boiler Scale. The early stages of preparation of the sample are shown under aluminum (page 242) after which the further treatment of the solution appears under calcium (page 590). Use 5 ml. of the solution so prepared, and determine magnesium hy Titan yellow.

Bones.[4] To 2 grams of ash, add sufficient 1:1 nitric acid to dissolve. Add an extra 2.0 ml. of 1:1 nitric acid and make up to 200 ml. To a 25-ml. aliquot in a 50-ml. volumetric flask add 15 ml. of water and heat to 80-90°. Add 5 ml. of 5 per cent ammonium molyhdate reagent, make up to volume, let stand for complete precipitation of phosphate, and filter. If less than 0.03 mg. of iron is present transfer 20 ml. of the filtrate to a 60-ml. centrifuge tnhe. If the filtrate contains more than 0.03 mg. of iron, transfer 40 ml. to a 60-ml. centrifuge tuhe and add 10 ml. of 1:1 ammonium hydroxide. Centrifuge and transfer 25 ml. of the supernatant liquid to another 60-ml. centrifuge tuhe. Whether separation of iron was necessary or not, add 3 ml. of concentrated ammonium hydroxide and 1 ml. of saturated ammonium carhonate solution and heat on a water hath. Centrifuge to separate the precipitate and use the supernatant liquid as sample for determination of magnesium hy 8-hydroxyquinoline.

Soil Extracts. *Total Magnesium.* Prepare a hydrochloric acid extract (page 642). Neutralize an aliqnot of the hydrochloric acid extract with 16 per cent sodium hydroxide solntion until a slight precipitate appears. Carefully add 1:9 hydrochloric acid drop hy drop until this

[3] Francesco Villani and Carlo Fariselli, *Met. ital.* 35, 10-12 (1943).

[4] G. Drouineau and A. Guédon, *Ann. agron.* 13, 421-6 (1943).

precipitate disappears. Precipitate iron and aluminum with a slight excess of 1:1 ammonium hydroxide. If there is considerable precipitate, redissolve and reprecipitate. Combine the filtrates and washings, dilute to a known volume, and take an aliquot for determination by Titan yellow.

Available magnesium. Evaporate an aliquot of a citric acid extract (page 643) of the soil to dryness and ignite. Dissolve the ash in the minimum amount of 1:5 hydrochloric acid and proceed as for total magnesium in soil extracts.

Replaceable Magnesium.[5] Percolate 10 grams of soil with 250 ml. of 8 per cent ammonium acetate. Transfer a 25-ml. aliquot to a 60-ml. centrifuge tube. Add 3 ml. of concentrated ammonium hydroxide and 1 ml. of saturated ammonium carbonate solution. Heat on a water bath for 10 minutes and centrifuge. The sample solution so obtained is suitable for precipitation and separation of magnesium as the hydroxyquinolate.

Biological Materials.[6] Transfer to a platinum dish a sample containing 0.05-3.0 mg. of magnesium as the oxide. Add 1 ml. of concentrated sulfuric acid and mix well. Evaporate to dryness on a steam bath and ash in a muffle at 500-600°, until the ash is white. Remove and, if tin is present, add 5-10 ml. of 1:2 nitric acid to dissolve and evaporate to dryness.

Water.[7] If the sample contains less than 2 ppm. of magnesium, acidify slightly with 1:3 hydrochloric acid and concentrate by evaporation in platinum. Aluminum will interfere and must be precipitated as the hydroxide.[8] Silica cannot be satisfactorily removed by sorption.

In either case, warm the sample with 1 ml. of concentrated hydro-

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[5] G. Drouineau and A. Guédon, *Ann. agron.* 13, 177-83 (1943); *ibid.* 15, 129-30 (1945); cf. W. Sherman Gillam, *Ind. Eng. Chem., Anal. Ed.* 13, 499-501 (1941); R. F. Reitemeier, *ibid.* 15, 393-402 (1943).

[6] P. E. Gregoire, *Arch. intern. physiol.* 43, 206-11 (1936); John P. Nielsen, *Ind. Eng Chem., Anal. Ed.* 11, 649-51 (1939); Christopher Carruthers, *ibid.* 15, 412-14 (1943).

[7] E. E. Ludwig and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* 14, 895-7 (1942); cf. R. Schmidt and G. Gad, *Kleine Mitt. Mitglieder, Ver. Wasser-, Boden- u. Lufthyg.* 13, 326 (1937); H. H. Müller-Neuglück, *Glückauf* 77, 34-7 (1941).

[8] Daniel J. Bengolea and Fortunato D. Amato, *Rev. obras. sanit. nación* (Buenos Aires) 11, No. 116, 71-8 (1947).

chloric acid, dilute with several volumes of water, and filter out silica, with or without tin as metastannic acid. Add a few drops of bromine water to the filtrate to oxidize the iron, boil to remove excess bromine, and bring to a pH of approximately 4.0 with 0.5 per cent sodium hydroxide solution, using bromocresol green as indicator. Make the final adjustment of the pH by buffering with 20 per cent sodium acetate solution. A slight excess of phosphate precipitates the iron at this pH. Filter the iron precipitate while hot and wash with distilled water. Add 1 ml. of saturated sodium oxalate solution, adjust the pH to 4.4-4.6 with 1 per cent oxalic acid, heat to boiling, and allow to stand for 2-3 hours to allow calcium to precipitate. Filter and wash with 1:50 ammonium hydroxide. Make the solution yellow with 1:50 hydrochloric acid, evaporate the filtrate to 10 ml., cool, and use as aliquot for the determination of magnesium by the 8-hydroxyquinoline method. The Titan yellow method may also be used in which case calcium need not have been separated.

Serum.[9] Obtain the serum within 2 hours from blood which shows no hemolysis. To 2 ml. of serum in a centrifuge tube, add 2 ml. of water and 1 ml. of 4 per cent ammonium oxalate solution. Centrifuge to remove precipitated calcium oxalate after 30 minutes. To 3 ml. of the clear upper layer, add 0.5 ml. of 5 per cent diammonium phosphate solution and make alkaline by dropwise addition of 0.25 ml. of concentrated ammonium hydroxide. Let the solution stand for 12 hours to complete precipitation of the magnesium ammonium phosphate. Centrifuge and decant the upper layer. Wash the precipitate thoroughly with water.

Dissolve the precipitate in 1 ml. of 1:2 sulfuric acid and 5 ml. of water and use this as sample for development of the molybdate and its reduction to molybdenum blue by stannous chloride. The phosphate may also be determined as the molybdivanadate[10] (page 672).

Alternatively,[11] to 2 ml. of serum add 2 ml. of water and 1 ml. of 4 per cent ammonium oxalate solution. Centrifuge after an hour. Filter and to the filtrate add with stirring an equal volume of 8 per cent trichloroacetic acid. Filter or centrifuge and use an aliquot of the clear upper layer for the determination of magnesium by the 8-hydroxyquino-

[9] Stig Borgström, *Skand. Arch. Physiol.* **78**, 64-72 (1938).

[10] Daisy G. Simonsen, Leola M. Westover and Maxine Wertman, *J. Biol. Chem.* **169**, 39-47 (1947).

[11] William S. Hoffman, *ibid.* **118**, 37-45 (1937).

line method. Titan yellow is also applicable to such a filtrate, in which case calcium need not be separated.[12]

One may also ash[13] 0.5-2.0 ml. of blood or serum in a muffle and extract with 1 ml. of 0.5 per cent acetic acid, followed by extraction with two 0.5-ml. portions. Combine the extracts and, to remove calcium add 1 ml. of a saturated solution of sodium oxalate, heat gently to boiling, remove, and allow to stand for 2-3 hours. Filter and wash with three 2-ml. portions of 1:50 ammonium hydroxide. Combine the washings and filtrate and use as aliquot for the determination of magnesium by precipitation by 8-hydroxyquinoline.

Urine.[14] To 4 ml. of albumin-free sample in a 15-ml. centrifuge tube, add 1 ml. of 4 per cent ammonium oxalate solution and 2 ml. of saturated sodium acetate solution. Mix, allow to stand for 30 minutes, and centrifuge. Use 6 ml. of the clear supernatant liquid as aliquot for the precipitation of magnesium as the phosphate and reduction by hydroquinone.

Alternatively,[15] make a 20-ml. sample alkaline with 20 per cent potassium hydroxide and boil to drive off ammonia. Cool and dissolve the precipitate in 1:4 acetic acid. Then add a small amount of 0.5 per cent uranyl acetate. Filter the precipitate into a 100-ml. volumetric flask and wash the precipitate with 1:100 acetic acid. Dilute the filtrate to volume for use of 5-ml. aliquots for determination by Titan yellow.

If the original sample contained albumin it must be ashed, the ash then being dissolved and calcium precipitated. Details given for milk are suitable.

Milk and Dairy Products.[16] Transfer 2 grams of milk solids to a platinum dish and ash in a muffle furnace. Dissolve the ash in 1:20 hydrochloric acid, wash into a beaker, and dilute to about 90 ml. Add a drop of thymol blue and add 1:20 hydrochloric acid dropwise until the solution turns red, showing that the pH is less than 1.2.

To remove calcium add 7 ml. of 6.4 per cent oxalic acid solution and

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[12] R. J. Garner, *Biochem. J.* 40, 828-31 (1947).

[13] J. W. Perish, L. L. Lachat and H. A. Halvorson, *Minn. Dept. Agr. Dairy and Food, Division Feed and Fertilizer Control*, March, 1936, 14 15; René Wolff, *Compt. rend. soc. biol.* 127, 1445 6 (1938).

[14] Carl Urbach, *Biochem. Z.* 241, 222-5 (1931).

[15] Carl Urbach, *Biochem. Z.* 252, 74-80 (1932); Carl Urbach and R. Baril, *Mikrochemie* 14, 343 61 (1934).

[16] J. H. Bushill, L. H. Lampitt and D. F. Filmer, *J. Soc. Chem. Ind.* 56, 411-13T (1937); G. T. Pyne, *Analyst* 68, 330 (1943).

heat to just below the boiling point. Add 1:15 ammonium hydroxide solution dropwise with stirring, over a period of 5 minutes until the solution is yellow or the pH is 3.0. Heat on a boiling water bath for 3 hours, cool, and filter within 30 minutes. Wash until free from chloride with ammonium oxalate solution. This is prepared by diluting 5 ml. of 6.4 per cent oxalic acid to 100 ml. and adjusting the pH to 3 with 1:12 ammonium hydroxide until the solution is yellow to thymol blue. Combine the filtrates and washings and use an aliquot for the determination of magnesium by precipitation as the phosphate and reduction to molybdenum blue.

Alternatively,[17] to 5 ml. of milk in a centrifuge tube add 0.5 ml. of an acetate buffer of 100 grams of sodium acetate and 30 ml. of glacial acetic acid diluted to 1 liter. Follow with 0.5 ml. of a 0.33 per cent solution of uranium acetate and 2.5 ml. of 95 per cent ethanol. Mix well and filter after 5 minutes. To 5 ml. of filtrate, add 3 drops of 1:10 ammonium hydroxide, 2 drops of 1:8 acetic acid, and 10 ml. of water warmed to 60-70°. Precipitate calcium with 1 ml. of 3 per cent ammonium oxalate and, after keeping the solution at 60° for 5 minutes, dilute to 20 ml. and centrifuge. Use 2-ml. aliquots of the clear solution for determination by Titan yellow.

Plant Tissue.[18] Ash 2 grams of dry plant tissue in a platinum or porcelain crucible. Dissolve the ash with 5 ml. of 1:1 hydrochloric acid. Warm on a hot plate to dissolve and, if necessary, add more hydrochloric acid. Dilute to a suitable volume, usually 100 ml., and centrifuge if necessary to eliminate turbidity due to silica.

To remove phosphate, transfer a suitable aliquot, usually 10 ml., to a centrifuge tube and add 1 ml. of 1 per cent lead acetate solution. Centrifuge to throw down the precipitate. Add 1 ml. of 1:35 sulfuric acid to precipitate lead and centrifuge again. Transfer the clear upper layer and add methyl red as indicator. Neutralize with 4 per cent sodium hydroxide solution. Add 2 ml. of 1:4 acetic acid and 5 ml. of 4 per cent ammonium oxalate solution to precipitate calcium. Let the precipitate stand overnight, filter, and wash.

Add to the filtrate and washings 2 ml. of 1:4 acetic acid and 3 ml. of a 5 per cent solution of 8-hydroxyquinoline in ethanol. Mix well and, if a precipitate of copper or nickel hydroxyquinolate forms, centrifuge

[17] Masayoshi Sato and Kiichi Murata, *J. Agr. Chem. Soc. Japan* **11**, 431-4 (1935).

[18] C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* **12**, 232-3 (1940); Monroe E. Wall, *Plant Physiol.* **15**, 537-45 (1940); cf. Omer J. Kelley, Albert S. Hunter and Athan J. Sterges, *Ind. Eng. Chem., Anal. Ed.* **18**, 319-22 (1946).

to separate it. Taking into consideration the reagents already present precipitate magnesium as the hydroxyquinolate in alkaline solution and later develop the color with ferric ion.

Alternatively ash and follow the technic described for bones (page 614). A method of preparation of plant material by wet ashing was given under potassium (page 550). An aliquot of the solution described there is a suitable sample. As another alternative the sulfur as sulfate, calcium, magnesium, sodium, and potassium are isolated as Solution C in determination of lead (page 30). Take a 10-ml. aliquot for precipitation with 8-hydroxyquinoline, finally determining by Folin's phenol reagent.

Grain and Oily Seeds.[10] Dry 20 grams of material at 100° and extract 4-5 times with anhydrous ethyl ether to remove fatty material which would react violently in the subsequent treatment. Add to the extracted material 20 ml. of concentrated nitric acid. Place on a steam bath and, if excessive frothing occurs, stir vigorously with a stirring rod. When the action subsides, cover with a watch glass to permit slow evaporation of the contents of the beaker to a thick yellow paste. Add 30-40 ml. of concentrated nitric acid and allow to digest on a steam plate for 6-7 hours or overnight. Take up the residue with 20 ml. of a mixture of 2 parts of nitric acid and 1 parts of perchloric acid and heat on an electric hot plate at 140-160° until the solution is clear. If the solution becomes dark, add concentrated nitric acid, a few ml. at a time. After the solution clears, remove the watch glass and allow excess perchloric acid to evaporate. To the cooled residue add 10 ml. of 1:1 hydrochloric acid, heat on a steam bath for 20 minutes, and filter into a 50-ml. volumetric flask. Wash 6 times with hot 1:9 hydrochloric acid and 4 times with water. Dilute to volume and use aliquots for the determination.

Standard

Dissolve 10.135 grams of magnesium sulfate heptahydrate in water and dilute to 1 liter. This contains 1 mg. of magnesium per ml. To prepare a standard from magnesium ammonium phosphate, dissolve 10.092 grams of air-dried salt in 100 ml. of 1:100 hydrochloric acid and dilute to 1 liter with water. Alternatively dissolve 1 gram of magnesium in 100 ml. of 1:100 hydrochloric acid and dilute to 1 liter with water. Each

[10] Martin E. Weeks and Jack R. Todd, *Ind. Eng. Chem., Anal. Ed.* **15**, 297-9 (1943).

ml. contains 1 mg. of magnesium. In any case for 0.1 mg. per ml. dilute 10 ml. to 100 ml.

Magnesium by 8-Hydroxyquinoline

Magnesium is precipitated by 8-hydroxyquinoline to form a light green hydroxyquinolate [20] of definite composition which is highly reproducible. Precipitation usually is carried out in hot ammoniacal solution. The complex may be filtered and the remaining excess of 8-hydroxyquinoline then determined [21] but that is not a usual technic.

The magnesium hydroxyquinolate is often dissolved in dilute hydrochloric acid [22] and the color read at 365 mμ, the color being stable for several days and following Beer's law.[23] It is more common to add ferric salts and determine by the green color of iron hydroxyquinolate in aqueous solution.[24] The concentration of ferric chloride has a substantial effect on the color produced. This is avoided by extracting the green-black reaction product with chloroform and diluting with butyl alcohol for comparison with standards.[25] Maximum light absorption for the ferric hydroxyquinolate occurs around 650-660 mμ with a secondary maximum at 425-460 mμ[26] Agreement with Beer's law is noted for 0.01-0.001 mg. per ml. The complex is also determined by Folin's phenol reagent.

If interfering elements such as silica, silver, copper, tin, iron, aluminum and calcium are eliminated, the method is very sensitive, accuracy being to 2 per cent. Calcium is generally precipitated as the oxalate and precipitation of magnesium may then take place without removal of the oxalate.[27]

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[20] M. Javillier and J. Lavollay, *Bull. soc. chim. biol.* 16, 1531-41 (1934); Trab. IX Congr. Internac. Química Pura y Aplicada, Tomo IV, Química Analitica, Madrid, 1934; J. Lavollay, *Bull. soc. chim. biol.* 17, 432-8 (1935).

[21] W. A. Hough and J. B. Ficklen, *J. Am. Chem. Soc.* 52, 4752-5 (1930).

[22] Makhlis, *Novosti Tekhniki, Ser. Gorno-Rudnayá Prom.* 1936, No. 25, 2; Rubens Salomé Pereira, *Rev. faculdade med. vet., Univ. São Paulo* 3, 83-99 (1945).

[23] Henry C. Deterding and Richard G. Taylor, *Ind. Eng. Chem., Anal. Ed.* 18, 127-9 (1946).

[24] J. Lavollay, *Bull. soc. chim. biol.* 17, 432-8 (1935); W. S. Hoffman, *J. Biol. Chem.* 118, 37-45 (1937); R. Wolff, *Compt. rend. soc. biol.* 127, 1445-6 (1938); C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* 12, 232-3 (1940).

[25] C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* 12, 232-3 (1940).

[26] D. L. Drabkin, *J. Assoc. Official Agr. Chem.* 22, 320 (1939); L. Gerber, R. I. Claassen and C. S. Boruff, *Ind. Eng. Chem., Anal. Ed.* 14, 658-61 (1942); Martin E. Weeks and Jack R. Todd, *ibid.* 15, 297-9 (1943).

[27] Michael Peech, *Ind. Eng. Chem., Anal. Ed.* 13, 436-41 (1941).

Procedure. *As Iron Hydroxyquinolinate in Aqueous Solution.* Transfer an aliquot containing 0.1-0.7 mg. of magnesium to a 15-ml. conical centrifuge tube. Add 5 drops of glacial acetic acid and 1 drop of methyl red indicator and mix thoroughly by tapping the outside of the tube with the fingers. Add 1:1 ammonium hydroxide dropwise with constant mixing from a buret until the solution turns faintly pink, indicating that the pH is about 6.0. Add 2-3 drops of 1:4 acetic acid to make the red more distinct. The pH of the final solution is 5.0-5.3.

Prepare a fresh reagent by dissolving 1 gram of 8-hydroxyquinoline in 50 ml. of 95 per cent ethanol. One ml. precipitates about 1.5 mg. of magnesium. If iron or aluminum is present, add 3 drops of reagent, mix, and place the tube in hot water for 10-15 minutes to precipitate iron and aluminum. Centrifuge while hot, pour the supernatant liquid into a similar tube, and wash the first tube carefully with warm water from a 2-ml. pipet. Stir up the precipitate, centrifuge, and transfer the washings to the second tube.

If calcium is present, precipitate by adding 1 ml. of a saturated solution of ammonium oxalate to the second tube and mixing thoroughly. Place the tube in a beaker of hot water and heat on a steam plate for 30 minutes. Remove from the heat and allow the tube to stand for 2-4 hours. Add 5-6 drops of 95 per cent ethanol to destroy any surface film of calcium oxalate and centrifuge. Transfer the supernatant liquid to a third centrifuge tube. Wash the calcium oxalate precipitate by pipeting 2-ml. portions of a 1:1 mixture of 95 per cent ethanol-1:9 ammonium hydroxide. Centrifuge and add the washings to the tube containing the magnesium solution.

To the third tube add 0.5 ml. of a 2 per cent 8-hydroxyquinoline solution in 95 per cent ethanol. If more than 0.7 mg. of magnesium is present, add 1 ml. Stir thoroughly with a rod, add 2 ml. of concentrated ammonium hydroxide, stir well, and remove the rod, washing with a fine stream of water. After a minute or two, when precipitation starts, add a layer of ethanol 1 cm. thick to prevent creeping of the precipitate up the sides of the tube. Addition of the alcohol too soon retards precipitation and, if it is added too late, it fails in its purpose. Place the tube in a beaker of hot water and keep on a hot plate for 20 minutes. Remove the tube from the bath, stopper with a rubber stopper, and allow to stand 1-2 hours or overnight. Do not permit the alcohol to evaporate entirely.

Centrifuge and draw off the liquid with gentle suction. Wash the precipitate with 2-ml. portions of a 1:1 mixture of 95 per cent ethanol and 1:9 ammonium hydroxide, covering the suspension each time with

a layer of alcohol. Centrifnge and draw off the supernatant liquid. Dry tbe precipitate carefully for a few minutes by placing the tube in a heaker of hot water on a steam hath, hut do not allow the precipitate to harden and become difficultly solnhle. Remove from tbe steam bath and add 10 ml. of a solution of 10 grams of ferric chloride hexahydrate in 2 liters of water containing 10 ml. of glacial acetic acid. Mix vigoronsly to break up the precipitate and hasten solution. It may be necessary occasionally to dissolve the warm precipitate first in 1:4 hydrochloric acid, cool, and then add the ferric chloride. This prevents tbe formation of a coating of iron hydroxyquinolate around the particles of the precipitate. Tbe color is fully developed for reading the transmittance in about 30 minutes. If the color is too dark, dilute with 10 ml. of the ferric chloride-acetic acid reagent, mix, and read.

As Iron Hydroxyquinolinate in Chloroform.[28] Take an aliquot containing 0.05-1.0 mg. of magnesium and dilute or concentrate to ahout 25 ml. If not already definitely acid, add 2 ml. of 1:4 acetic acid and mix. Add 3 ml. of a 5 per cent solution of 8-hydroxyquinoline in ethanol. If any precipitate separates, centrifuge or filter and discard the precipitate.

Add 5 ml. of concentrated ammoninm hydroxide and mix well. Stopper loosely and heat in a boiling water hath for 30 minutes. Cool and add 5 ml. of chloroform. Shake at intervals over a period of an hour to extract the hydroxyquinolinates of iron, manganese, aluminum, etc., into the chloroform layer. The magnesium hydroxyquinolate is not so extracted. Filter on a fritted-glass filter. Wash tbe precipitate witb 5 ml. of 1:2 ammonium hydroxide, then with chloroform until no more green-black color is extracted, using at least 2 ml.

Dissolve tbe washed precipitate of magnesium hydroxyquinolate in 5 ml. of 1:1 hydrochloric acid, using a 50-ml. volumetric flask as receiver. Wash the filter with water and dilute to volume. Transfer a suitable aliquot of the solntion, such as 10 ml., to a separatory funnel and add 3 ml. of a 1 per cent solution of ferric chloride bexahydrate and 3 ml. of 50 per cent sodium acetate solution. Mix well and add 5 ml. of chloroform. Shake well and remove the chloroform layer for later use. Extract with 2-ml. portions of chloroform until the extracts are no longer colored and dilute the combined chloroform extracts with butyl alcohol to a known volume. Compare with a standard of similar color intensity.

[28] C. P. Sideris, *ibid.* 12, 232-3 (1940).

As Magnesium Hydroxyquinolate in Acid Solution. Dilute a neutral aliquot of sample containing 3-10 mg. of magnesium to about 100 ml. Add 5 ml. of a 2 per cent solution of 8-hydroxyquinoline in isopropyl alcohol, plus 2 ml. of the 8-hydroxyquinoline solution for every mg. of magnesium above 3 mg. Add 10 ml. of concentrated ammonium hydroxide and heat to a vigorous boil. Decrease the heat to just below boiling for 5-10 minutes to allow the precipitate of magnesium hydroxyquinolate to settle. Remove from the heat and allow to stand for 5 minutes. Filter and wash with a solution made up of 50 ml. of isopropyl alcohol and 50 ml. of 1:25 ammonium hydroxide. Discard the filtrate and washings. Dissolve the precipitate in 40 ml. of hot 1:1 hydrochloric acid and wash with hot water, using a 200-ml. volumetric flask as receiver. Cool and dilute to volume. If the magnesium content is high, transfer a 20-ml. aliquot to a second flask, add 10 ml. of 1:1 hydrochloric acid, and dilute to volume. Measure the transmittance at 365 mμ or compare with standards.

By Reduction of Phenol Reagent. The Folin phenol reagent consists of a mixture of 100 grams of sodium tungstate, 20 grams of phosphomolyhdic acid, 50 ml. of 85 per cent phosphoric acid, and 750 ml. of water, boiled together for 2 hours and diluted to 1 liter. Transfer a sample containing 0.005-0.05 mg. of magnesium to a 25-ml. volumetric flask and a similar standard to a similar flask. To each add 5 ml. of sodium carbonate solution and dilute to about 23 ml. Add 1 ml. of phenol reagent to each, mix, and immerse in a boiling water bath for about 30 seconds. Cool to room temperature, dilute to volume, and compare.

Magnesium by Titan Yellow

When magnesium hydroxide is precipitated by sodium hydroxide in the presence of the sodium salt of dehydrothio-*p*-toluidine sulfonic acid, or Titan yellow, a red-orange to red color forms.[29] The dye, of which there should always he an excess, and the lake are in equilibrium and follow the laws of adsorption. The intensity of color reaches a maximum, instead of increasing indefinitely.[30] No definite compound is formed and, as would be predictable, the system does not conform to Beer's law.

Substantial amounts of iron, manganese, aluminum, silicon, and

[29] H. D. Barnes, *J. South African Chem. Inst.* **11**, 67-8 (1918); I. M. Kolthoff, *Biochem. Z.* **185**, 344-8 (1927); *Chem. Weekblad* **24**, 254-5 (1927); Jan Bečka, *Biochem. Z.* **233**, 118-28 (1931).

[30] H. Ginsberg, *Z. Elektrochem.* **45**, 829-33 (1939).

organic compounds, such as proteins, must be absent. Tin and zinc can be precipitated with ammonium sulfide. The concentration of ammonium salts should be kept at a minimum. Concentrations of aluminum and tin as low as 4 ppm. and of phosphate in excess of 100 ppm. reduce the color intensity and produce low readings.[31] Calcium does not interfere unless its concentration exceeds 500 ppm. Amounts of magnesium up to 0.015 mg. are estimated photometrically with an accuracy of 0.0001 mg. by use of a light-green or yellow-green filter. An orange color results with 1 mg. of magnesium per liter, a red color with 5 mg. per liter, and a red flocculent precipitate with a greater concentration of magnesium. The period of stability appears to be about 1.5 hours for 4 mg. per liter, 4 hours for 3 mg., and at least 12 hours for the more dilute solutions. A minimum of 0.2 mg. per liter is detectable.

The addition of a protective colloid such as agar,[32] starch,[33] gum ghatti,[34] or dextrin increases the stability and makes the readings highly reproducible, whereas the presence of calcium ion deepens the red color.[35] The minimum transmittance is at 525-530 mμ.

Procedure. *Oxidizing Agents Absent.*[36] Measure out an aliquot of sample which will contain 0.1-1.0 mg. of magnesium. If the volume exceeds 40 ml., add a few drops of 1:1 hydrochloric acid and evaporate in platinum. For smaller volumes dilute to about 40 ml. Transfer to a 100-ml. volumetric flask.

Add, with mixing after each addition, 1 ml. of 1:35 sulfuric acid, 20 ml. of saturated calcium sulfate solution, 10 ml. of a 0.05 per cent solution of Titan yellow, and 10 ml. of clear 8 per cent sodium hydroxide solution containing 10 per cent of gum arabic. Dilute to volume, pour into a larger flask, and shake vigorously for 5 minutes. Let the solution stand for separation of air bubbles. In the meantime set the photometer at 100 with the same mixture in which distilled water has been substituted for the sample. Read at 500 or 525 mμ.

Oxidizing Agents Present.[37] The sample must be substantially free

[31] W. Sherman Gillam, *Ind. Eng. Chem., Anal. Ed.* 13, 499-501 (1941).

[32] Carl Urbach, *Biochem. Z.* 252, 74-80 (1932).

[33] E. E. Ludwig and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* 14, 895-7 (1942).

[34] R. J. Garner, *Biochem. J.* 40, 828-31 (1947).

[35] R. Schmidt and G. Gad, *Kleine Mitt. Mitglieder Ver. Wasser-, Boden- u. Lufthyg.* 13, 326 (1937).

[36] E. E. Ludwig and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* 14, 895-7 (1942).

[37] W. Sherman Gillam, *ibid.* 13, 499-501 (1941).

from iron, aluminum, and phosphate. Select an aliquot containing 0.03-0.3 mg. of magnesium. Evaporate to dryness and ignite gently, thus volatilizing accumulated ammonium salts. Take up in water, if necessary adding a drop or two of 1:1 hydrochloric acid. Transfer to a 100-ml. volumetric flask and add 10 ml. of 5 per cent sucrose solution and 2 ml. of a 4 per cent solution of hydroxylamine hydrochloride. Prepare a solution containing 0.15 gram of Titan yellow in 75 ml. of 95 per cent ethanol, made up to 100 ml. with water. Add 0.5 ml. of this reagent and dilute to about 70 ml. Add 10 ml. of 4 per cent sodium hydroxide solution and dilute to volume. Mix well and read the transmittance with the zero point set with 10 ml. of 4 per cent sodium hydroxide and 0.50 ml. of Titan yellow reagent per 100 ml.

Magnesium as Molybdenum Blue

Magnesium in ammoniacal solution treated with the mono- or dibasic phosphate forms magnesium ammonium phosphate.[38] The salts present and the temperature of the precipitation affect the rate of precipitation and the nature of the precipitate. If the precipitate is of a suspended nature, obtained by vigorously rubbing the sides of the vessel, fairly consistent results are obtained. If, however, a sediment is deposited on the sides of the vessel, high values are obtained, probably due to the coprecipitation of some $Mg(NH_4)_4(PO_4)_2$ instead of the usual $MgNH_4PO_4 \cdot 6H_2O$.

In the precipitation of magnesium as magnesium ammonium phosphate, manganese is coprecipitated.[39] If manganese is present, dissolve the precipitate in 1:5 sulfuric acid, neutralize with 20 per cent sodium hydroxide solution, and add a few ml. of 50 per cent sodium acetate solution as a buffer. Precipitate manganese as the oxide by heating with bromine water, filter, boil off the bromine from the filtrate, and then reprecipitate magnesium as the phosphate.

Determining magnesium by the phosphate method is not completely satisfactory because magnesium ammonium phosphate solutions tend to remain supersaturated, making complete precipitation of minute amounts difficult. The procedure is longer than that with 8-hydroxyquinoline.

Having separated the magnesium ammonium phosphate, magnesium is then determined indirectly by dissolving the precipitate and determin-

[38] R. F. Reitemeier, *ibid.* **15**, 393-402 (1943); Omer J. Kelley, Albert S. Hunter and Athan J. Sterges, *ibid.* **18**, 319-22 (1946).

[39] J. Duckworth and W. Godden, *Analyst* **63**, 805-9 (1938); C. Karunakaran and K. Neelakantam, *Proc. Indian Acad. Sci.* **24A**, 448-50 (1940).

ing the amount of phosphate present. In general, all methods applicable to phosphates, such as stannous chloride, aminonaphtholsulfonic acid, or hydroquinone may be used. If the magnesium content is high, then estimation as the yellow phosphomolybdate is preferable to reduction to molybdenum blue.

Procedure. To an aliquot of sample containing 0.1-1.0 mg. of magnesium add 2 ml. of 7.5 per cent potassium dihydrogen phosphate in 1:2.5 sulfuric acid. Ash slowly in a platinum dish over an open flame to remove ammonium salts. Avoid excessive heating. Add 10 ml. of 1:11 hydrochloric acid to the residue and evaporate to dryness. Cool, and dissolve, with stirring, in 5 ml. of 1:100 hydrochloric acid.

To this solution add 3 drops of 10 per cent ammonium chloride, 1 ml. of 3 per cent disodium phosphate heptahydrate, and 1 drop of 0.5 per cent phenolphthalein solution. Add sufficient 1:9 ammonium hydroxide to make the solution pink and a drop in excess. Scratch the inside walls with a glass rod for 1 minute to assist in precipitation of magnesium ammonium phosphate. Set aside for about 18 hours at 0-5°. Filter through a glass filter, wash with four 5-ml. portions of 1:100 ammonium hydroxide, and follow with three 10-ml. portions of 96 per cent ethanol.

Take up the precipitate in 2 ml. of 1:10 hydrochloric acid and wash the filter with successive amounts of hot water. Evaporate the filtrate and washings on a steam bath and cool. Dissolve the residue in exactly 10 ml. of 1:35 sulfuric acid and determine phosphorus in a 2-ml. portion by the stannous chloride method (page 668). Alternatively, use other reducing agents.

MISCELLANEOUS

The sorption of iodine by magnesium hydroxide from a hydroiodate solution gives a brown color which measures the magnesium. One technic is to treat with a mixture of 14 ml. of a 0.4 per cent solution of sodium hydroxide and 16 ml. of 1.27 per cent iodine solution, and compare with standards.[40] Silica should be removed by filtration, and aluminum, iron, and manganese precipitated with freshly prepared barium carbonate. A similar reaction is obtained by treating the solution with a sodium hypochlorite-potassium iodide mixture.[41] Sodium hypobromite and potassium iodide are also used.

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[40] A. K. Babko, *Zavodskaya Lab.* **4,** 518-20 (1935).

[41] Virgilio Lucas, *Rev. assoc. brasil. farm.* **17,** 9-16 (1936).

The magnesium salt of the dye tropeolin 00 is insoluble in water and forms a red-violet solution in acid that lends itself to the colorimetric measurement of magnesium.[42] For determination in serum add 2 ml. of half-saturated ammonium oxalate to a 2-ml. sample. After 1 hour centrifuge the precipitated calcium oxalate. To deproteinize the upper layer, separate and add 2 ml. of 10 per cent sodium tungstate solution, add 2 ml. of 1:50 sulfuric acid, and dilute to 10 ml. Mix well and filter. Transfer 4 ml. of the filtrate to a 10-ml. tube and heat in boiling water. Add 2 ml. of freshly filtered saturated solution of tropeolin 00. Mix, cool in ice water, and centrifuge after 1 hour. The solubility of the magnesium salt of tropeolin 00 is about 1 part in 10 million. Wash with 4-ml. portions of water until the washings show only a trace of color. Dissolve the precipitate in 4 ml. of cold concentrated sulfuric acid and transfer quantitatively to a 50-ml. flask. Use water for the transfer and dilute to volume. Read the transmittance around 530 mμ or balance against a standard.

Since tropeolin 00 reacts with both calcium and magnesium,[43] by use of a series of standards a 0.4 per cent solution in 50 per cent ethanol is suitable for use in determination of hardness of water with a series of standards.

When quinalizarin is added to magnesium in alkaline solution, there is a color change from violet to indigo blue.[44] The addition of gum arabic solution helps to prevent precipitation. Beryllium forms the same color but it disappears on addition of sodium hydroxide.[45] When ammonium hydroxide is added, the color due to magnesium disappears, whereas that due to beryllium persists. Aluminum does not react.

Another dye which will detect 0.001 mg. per ml. of magnesium in alkaline solution is 1,2,5,8-tetrahydroxyanthraquinone, the color changing from red-violet to light blue.[46] Large amounts of aluminum must have been removed. Calcium does not interfere. Likewise, sorption of yellow thiazole J by magnesium hydroxide causes a color change from yellow to rose at pH 11.[47]

Curcumin forms a yellow to orange lake with magnesium which is

[42] Konrad Lang, *Biochem. Z.* **253**, 215-17 (1932); R. Nordbo, *Skand. Arch. Physiol.* **81**, 265-8 (1939).

[43] S. M. Drachev, *Zavodskaya Lab.* **11**, 46-8 (1945).

[44] A. Thiel and Eitelfriedrich van Hengel, *Ber.* **71B**, 1157-62 (1938).

[45] Giovanni Venturello, *Ricerca sci.* **14**, 256 60 (1943).

[46] F. L. Hahn, *Mikrochem. Pregl Festschr.* 127-39 (1929).

[47] Gaston Charlot, *Bull. soc. chim.* **8**, 220-2 (1941); Rene Legendre, *Compt. rend. soc. biol.* **136**, 291-2 (1942).

suitable for colorimetric estimation.[48] Phosphates affect the color and suggest that the product is a magnesium phosphate-curcumin lake. Iron should be removed as its color will interfere. Borates up to 8 mg. do not alter the color. The suspension is desirably stabilized by a solution such as that of starch glycerite. Calcium need not be absent and phosphates should be present. Dilute an aliquot to about 40 ml. Add 2 ml. of a filtered aqueous solution of starch glycerite. Mix well and add accurately 4 drops of 1 per cent alcoholic solution of curcumin. Mix and add 5 ml. of 16 per cent sodium hydroxide solution. Mix well, dilute to volume, and mix. Compare with standards prepared at the same time in the same way from a standard magnesium solution containing phosphates.

The sum of calcium and magnesium present may be determined by reaction with ferrocyanide.[49] Calcium may be determined in the presence of magnesium as the ricinoleate. The difference between these determinations is the amount of magnesium present.

Magnesium alone may be estimated nephelometrically or turbidimetrically as the phosphate after removal of interfering elements.[50] To duplicate cylinders add 10 ml. of 0.2 N trisodium phosphate solution and 10 ml. of 1:9 ammonium hydroxide and dilute to 50 ml. To the first cylinder add 10 ml. of sample, dropwise with stirring. Dilute to 80 ml. To the other cylinder add standard magnesium solution until the solutions match, either nephelometrically or turbidimetrically.

The procedure for calcium as the oleate may be applied to magnesium[51] in the absence of calcium. Dilute the sample in a 50-ml. volumetric flask to about 45 ml. In a similar flask take a solution containing a known amount of magnesium. To each add 2 ml. of a solution containing 100 grams of ammonium chloride and 10 ml. of concentrated ammonium hydroxide per liter. Mix and add 1 ml. of a solution of 2 grams of oleic acid and 0.5 gram of potassium hydroxide per liter of 60 per cent ethanol. Dilute each to 50 ml., mix, and let stand for 2 hours. Compare the pale yellow nephelometrically.

Magnesium is determined in the same manner as calcium by the reduction in color of ferric thiocyanate. The method is accurate to about

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[48] I. M. Kolthoff, *J. Am. Chem. Soc.* **50,** 395 (1928); W. E. Thrun, *Ind. Eng. Chem. Anal. Ed.* **2,** 8 (1930); *ibid.* **4,** 426-7 (1932); F. Thompson, *Ind. Chemist* **10,** 142 (1934).

[49] Fritz Feigl and F. Pavelka, *Mikrochemie* **2,** 85-91 (1924); Leonia Kriss, *Biochem. Z.* **162,** 359-65 (1925).

[50] E. V. Vasil'eva, *Zavodskaya Lab.* **1933,** No. 8, 10-13.

[51] A. Gregoire and T. Sola, *Bull. soc. chim. Belg.* **32,** 131 (1923).

5 per cent.[52] To the sample or an aliquot add exactly 2 ml. of ferric thiocyanate solution containing 5 ml. of 0.3 per cent ammonium thiocyanate solution, 5 ml. of 0.3 per cent ferric chloride solution and sufficient 1:1 hydrochloric acid to clarify the solution, diluted with water to make 50 ml. It should be at least one-half hour old before use. Dilute the sample to 10 ml. with 1:60 hydrochloric acid and compare lengthwise with a series of standards prepared in the same way. The color is inversely, rather than directly, proportional to the magnesium content.

Magnesium hydroxyquinolate may be colored by coupling with a dye, such as diazobenzenesulfonic acid, permitting quantitative estimation within the range 0.01-0.5 mg. of magnesium.[53]

[52] W. M. Marriott and J. Howland, *J. Biol. Chem.* 32, 233-9 (1917); B. Kramer and F. F. Tisdall, *ibid.* 48, 223-32 (1921).

[53] F. Alten, H. Weiland and H. Loofmann, *Angew. Chem.* 46, 668-9 (1933); F. Alten, H. Weiland and B. Kurmies, *ibid.* 46, 697-8 (1933).

CHAPTER 46

PHOSPHORUS

THE DISTRIBUTION of small amounts of phosphorus is so wide—metals and alloys, minerals, biological samples—that there are necessarily correspondingly wide needs for methods. In general it is converted to orthophosphate for determination. Metaphosphate and pyrophosphate are also determinable as such. If no orthophosphate is present, it is more convenient to hydrate them to that form and so determine them.

Phosphatase may be determined as the phosphate, or the phenol liberated. This chapter considers only the first of these, and when determined by the liberated phenol the methods will appear under phenol in the third volume (in preparation).

The outstanding method for determination of orthophosphate is as molybdenum blue derived by reduction of the phosphomolybdate or the more recent phosphovanadomolybdate. A secondary type is the yellow color due to phosphovanadomolybdate or phosphomolybdate without reduction. In general silica and arsenic react much the same, leading to the necessity of separations for many determinations. The tendency in metal analysis is to remove or inactivate arsenic and silicon and then use the molybdenum blue method, commonly with stannous chloride reduction. A great variety of reducing agents are available ranging from hydroquinone to 1-amino-2-naphthol-4-sulfonic acid.

SAMPLES

Air. Determine phosphine [1] in air by oxidation to orthophosphoric acid. Bubble the necessary volume of gas through 3 absorption flasks in series, each containing 10 ml. of 0.025 per cent potassium permanganate solution and 1 ml. of 1:20 sulfuric acid. Combine the solutions, add 10 ml. of 0.05 per cent oxalic acid or 0.06 per cent sodium oxalate solution, heat until decolorized, and use an aliquot. Reduce the orthophosphate to molybdenum blue with hydroquinone.

Determine phosphorus oxychloride [2] similarly by absorbing in water and determine the resulting orthophosphoric acid by reduction to molybdenum blue.

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[1] W. Müller, *Arch. Hyg. Bakt.* **129,** 286-92 (1943).

[2] O. D. Khalizova, *Zavodskaya Lab.* **8,** 940-3 (1939).

Determine phosphorus vapor in air by absorption in bromine water. Evaporate the solution and determine total phosphorus as orthophosphate in an aliquot of the solution.

Absorb phosphorus trichloride in 3 per cent silver nitrate solution for fixation. Oxidize the silver phosphide with bromine water and determine the phosphorus trichloride as orthophosphoric acid by reduction to molybdenum blue.

Acetylene.[3] Pass a known amount of sample through a series of wash bottles containing a 1 per cent solution of sodium hypochlorite. Phosphine is oxidized to orthophosphoric acid and hydrogen sulfide to sulfuric acid. Combine the washings and acidify with 5 ml. of 1:1 hydrochloric acid. Evaporate to dryness and take up in water. Dilute to a known volume. Use an aliquot for determination of phosphorus by reduction to molybdenum blue with hydroquinone.

Combustible Gases. Mix the gas with oxygen and ignite by an electric discharge. Collect the water formed and determine phosphorus as orthophosphoric acid by reduction to molybdenum blue.

White Phosphorus.[4] Add water to cover the sample which will prevent loss by evolution of phosphorus bromides, then excess bromine in carbon tetrachloride. Heat until the carbon tetrachloride has been volatilized along with excess of free bromine. Neutralize to litmus and dilute to a known volume for the use of aliquots by ammonium molybdate and hydroquinone reduction.

Iron and Steel. *Cast Iron.*[5] Dissolve a 0.1-gram sample in 25 ml. of 1:1.6 nitric acid. Boil to expel nitrous fumes, cool, and dilute volumetrically to 100 ml. Allow the graphite to settle. Pipet out a 5-ml. aliquot and add 3 ml. of 60 per cent perchloric acid. Evaporate the solution to fumes and heat gently until all the nitric acid has been removed. If arsenic is present to not over 0.25 per cent, add 5 ml. of 1:4 hydrobromic acid to the cooled perchloric acid solution of the sample, evaporate to fumes, and fume gently to volatilize the hydrobromic acid carrying with it arsenic tribromide. Again cool, dilute to a known volume,

[3] S. S. Perel'man and T. M. Lelyakina, *Zavodskaya Lab.* **11**, 810-14 (1945).

[4] W. Harka, *Mikrochemie ver. Mikrochim. Acta* **32**, 127-32 (1944).

[5] John L. Hague and Harry A. Bright, *J. Research Natl. Bur. Standards* **26**, 405-13 (1941); cf. J. B. Fortune *et al.*, *J. Iron Steel Inst.* (London), Advance copy, Nov. 1943, 16 pp.

and use an aliquot for determination as molybdenum blue by hydrazine reduction. In sulfuric acid solution after similar volatilization of arsenic, the color may be developed as the vanadomolybdate.[6]

As another method of oxidation,[7] treat a nitric acid solution of sample with 5 ml. of 2 per cent potassium permanganate, boil until the color disappears, and add 5 ml. of 3 per cent ammonium chromate solution. Boil until the color again disappears, dilute volumetrically to 100 ml., and use a 2-ml. aliquot. Develop molybdenum blue with stannous chloride but extract the phosphomolybdate with ether before reduction.

Carbon and Alloy Steel.[8] This method of solution given for cast iron is also applicable without modification to alloy steels containing less than 2 per cent of chromium, 5 per cent of vanadium, or 35 per cent of nickel. If the sample is insoluble in 1:1.6 nitric acid, add 3 ml. of 1:1 hydrochloric acid to the nitric acid. Heat 50 mg. of sample with 5 ml. of 1:1.6 nitric acid, adding hydrochloric acid if necessary. Cool and pipet in 3 ml. of 60 per cent perchloric acid. If silicon is present in large amounts, add 2 drops of 48 per cent hydrofluoric acid. Evaporate to fumes of perchloric acid and fume gently for 3-4 minutes to remove nitric acid. Cool and use an aliquot for reduction to molybdenum blue by hydrazine. If the steel contains more than 2 per cent of chromium, 5 per cent of vanadium, or 35 per cent of nickel, run a blank in which 1:12.5 sulfuric acid is substituted for the reagents in the procedure, dilute to volume with water, and apply the correction so obtained. Errors[9] due to the presence of tungsten, columbium, chromium, copper, vanadium, or nickel in steel disappear if carbides are decomposed by prolonged heating with perchloric acid and oxides of columbium and tungsten are removed by filtration.

As another technic dissolve 0.5 gram of sample in 20 ml. of 1:2 nitric acid and allow any violent reaction to subside.[10] Heat to boiling on a hot

[6] W. M. Murray, Jr. and S. E. Q. Ashley, *Ind. Eng. Chem., Anal. Ed.* 10, 1-5 (1938); G. Bogatzki, *Arch. Eisenhuttenw.* 12, 539-42 (1938); V. D. Konkin, *Zavodskaya Lab.* 8, 322-4 (1939); M. G. Mellon, *Proc. Am. Soc. Testing Materials* 44, 772-3 (1944).

[7] I. F. Drozd, *Novosti Tekhniki* 1936, No. 44, 28.

[8] John L. Hague and Harry A. Bright, *J. Research Natl. Bur. Standards* 26, 405-13 (1941); W. B. Sobers, *Am. Foundryman* 6, No. 9, 2-4 (1944); cf. Max Herzog, *Chemist-Analyst* 33, 4-7 (1944).

[9] Henry L. Katz and Kenneth L. Proctor, *Anal. Chem.* 19, 612-14 (1947).

[10] R. E. Kitson and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 16, 379-83 (1944); M. G. Mellon, *Proc. Am. Soc. Testing Materials* 44, 772-3 (1944); cf. V. D. Konkin, *Zavodskaya Lab.* 8, 322-4 (1939).

plate and boil for 2 minutes to remove nitrogen oxides. If graphite is present, filter immediately through an inorganic filter to prevent decolorization of the phosphovanadate. Add 5 ml. of 7.5 per cent ammonium persulfate solution and boil for 3-5 minutes to destroy excess oxidizing agent. Cool and use for the determination of phosphorus as the phosphovanadomolybdic complex.

In determination of phosphorus in steel the transmittance varies with temperature, provided the iron salt has not been removed.[11] When iron is absent, the temperature effect is negligible. The best way of avoiding this complication is to use a blank of the sample instead of a water blank in setting the instrument and to use blank and developed sample at the identical temperature.

Stainless Steel.[12] Heat a 0.05-gram sample with 5 ml. of concentrated nitric acid and 5 ml. of concentrated hydrochloric acid until solution is complete. Add 4 ml. of 72 per cent perchloric acid and evaporate until vapors of perchloric acid condense in the neck of the flask and the chromium is oxidized to chromic acid. Cool, dilute with 10 ml. of water, and use for the determination by reduction with hydrazine sulfate to molybdenum blue.

Alternatively,[13] dissolve a 0.2-gram sample in 3 ml. of concentrated nitric acid and 2 ml. of concentrated hydrochloric acid. Evaporate to dryness. Add 3 ml. of concentrated nitric acid and again evaporate to dryness. Repeat the evaporation to dryness. Take up in 5 ml. of water and add 2 drops of 1 per cent silver nitrate solution, 2 ml. of 1:1 sulfuric acid, and 3 grams of ammonium persulfate. Boil for 10 minutes, then add 1 ml. of 1:1 hydrochloric acid. Add 1:1 ammonium hydroxide until ferric hydroxide and ferric phosphate are precipitated. Add 1:1 hydrochloric acid until the hydroxide and phosphate are dissolved and filter. Use as a sample for development of the molybdenum blue color.

Ferrovanadium.[14] Add 40 ml. of 1:2 nitric acid to a 0.1 gram sample. When solution is complete, add 40 ml. of 7.5 per cent ammonium persulfate solution and boil until the persulfate is decomposed. Let cool, add 40 ml. of 20 per cent ferrous sulfate solution to reduce the vanadium, and dilute to 200 ml. Use 2 ml. as sample for reduction to molybdenum

[11] Uno T. Hill, *Anal. Chem.* **19**, 316-19 (1947).
[12] W. J. Boyer, *Proc. Am. Soc. Testing Materials* **44**, 774-6 (1944).
[13] A. I. Kokorin, *Zavodoskaya Lab.* **12**, 125-7 (1946).
[14] N. D. Ivanova and S. I. Malov, *ibid.* **12**, 246-8 (1946).

blne with stannous chloride, extracting the blue into ether for comparison.

Ferromanganese. Follow the technic for ferrovanadium but omit the addition of ferrous sulfate solution.

Ferromolybdenum. Dissolve a 0.5-gram sample in 40 ml. of 1:2 nitric acid. Add 40 ml. of 7.5 per cent ammonium persulate solution and boil until the persulfate is decomposed. Add 10 ml. of 2.5 per cent potassium permanganate solution and boil for 5 minutes. Add 6 per cent ammonium oxalate or oxalic acid solution until the permanganate is decolorized and manganese dioxide is in solution. Dilute to about 200 ml. and add 1:1 ammonium hydroxide until the solution is alkaline with ferric hydroxide and phosphate precipitated. Filter, wash with hot water, and discard the filtrate. Dissolve the precipitate in 20 ml. of hot 1:2 nitric acid, using the flask in which it was precipitated as receiver. Repeat the precipitation and solution of the precipitate. Dilute to 100 ml. and use 2 ml. as aliquot for development of molybdenum blue by stannous chloride reduction. An alternative method of decolorizing the excess permangate is with a saturated solution of sulfur dioxide.[15]

Ferrotitanium. Dissolve a 1-gram sample in 40 ml. of 1:2 nitric acid. Add 40 ml. of 7.5 per cent ammonium persulfate solution and boil until the persulfate is decomposed. Add 10 ml. of 25 per cent potassium permanganate solution and boil for 5 minutes. Add 6 per cent oxalic acid solution until the permanganate is decomposed and the manganese dioxide is dissolved. Dilute to about 100 ml. and pour into 250 ml. of 25 per cent sodium hydroxide solution, with stirring. Heat to boiling, cool, and dilute to 500 ml. Filter, neutralize the filtrate with 1:2 nitric acid, and add 20 ml. in excess. Add 30 ml. of hot 5 per cent ammonium alum solution, then 1:1 ammonium hydroxide to precipitate aluminum hydroxide and phosphate. Heat to boiling and filter. Wash the precipitate on the filter and discard the filtrate. Dissolve the precipitate from the filter with 20 ml. of hot 1:2 nitric acid, using a 100-ml. volumetric flask as receiver. Dilute to volume and use a 2-ml. aliquot for development of molybdenum blue by stannous chloride reduction.

Nickel-chrome Alloys. A solution was prepared for determination of chromium (page 267) of which an aliquot may be used for phosphorus.

[15] T. S. Harrison and W. Fisher, *J. Soc. Chem. Ind.* 62, 219-21 (1943); T. S. Harrison, *ibid.* 63, 350-1 (1944); cf. Kurt A. F. Schmidt and Karl Kutil, *Stahl u. Eisen* 64, 539-40 (1944).

Copper Alloys.[16] If the phosphorus content is 0.01-0.2 per cent use a 1-gram sample of drillings or sawings. For 0.06-1.2 per cent of phosphorus use a 0.5-gram sample. Accurately mix 32 ml. of concentrated nitric acid and 12 ml. of concentrated hydrochloric acid with 50 ml. of water and dilute to 100 ml. Add 20 ml. of this mixed acid and a few grains of silicon carbide to the sample. Heat gently until dissolution is complete. Add 1 ml. of 3 per cent hydrogen peroxide, prepared by dilution of the 30 per cent grade, and boil gently for 5 minutes. Stabilizers added to the commercial 3 per cent grade render it unsuitable. Avoid vigorous boiling as loss of acid will affect the subsequent development of color. Remove from the source of heat and cool. Transfer to a 100-ml. volumetric flask. Use the entire solution for development of the color as the phosphovanadomolybdate.

Deoxidized Copper.[17] Use a 1-gram sample and, as a blank, an exactly equal portion of high-purity electrolytic copper. To each beaker, add 10 ml. of 2:3 nitric acid. Cover and let stand on a steam bath until completely dissolved. Boil for 1 minute to expel oxides of nitrogen but avoid excessive boiling as loss of acid will affect the color development. Add 2 ml. of 1 per cent potassium permanganate solution and heat just to boiling. Add 1 ml. of 3 per cent hydrogen peroxide prepared by dilution of the 30 per cent grade. Swirl until the solution clears and boil gently until all excess hydrogen peroxide has been destroyed. Cool and transfer to a 100-ml. volumetric flask. Use the entire solution for development as the phosphovanadomolybdate.

Phosphorized Brass.[18] Use a 1-gram sample of copper and an exactly equal amount of high-purity electrolytic copper as a blank. Add 10 ml. of 2:3 nitric acid and an additional 0.7 ml. for each additional 100 mg. if the total weight exceeds 1 gram. Warm on a steam bath until decomposed and complete as for deoxidized copper starting at "Boil for 1 minute to expel oxides of nitrogen . . .".

Vanadium Slag.[19] Heat a 0.5-gram sample with 1 gram of magnesium oxide, 0.5 gram of sodium carbonate, and 0.15 gram of potassium nitrate in a current of oxygen to 1000°. Cool, moisten with water, and

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[16] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 335-7. American Society for Testing Materials, Philadelphia, Pa.

[17] *Ibid.*, pp. 334-5.

[18] *Ibid.*, pp. 334-5.

[19] B. Ya. Barkov, *Zavodskaya Lab.* 12, 627-9 (1946).

treat with 10 ml. of 1:1 hydrochloric acid. Heat until all sodium salts are dissolved, filter, and wash with 1:50 hydrochloric acid on the filter. Finally dilute to a suitable volume and take an aliquot for reduction to molybdenum blue.

Iron Ores.[20] Determination of phosphorus in iron ores is carried out satisfactorily by this method if the iron content is not below 50 per cent. Below that there is marked reduction in the values obtained. The use of perchloric acid changes iron to the colorless ferric perchlorate.

To a 1-gram sample add 10 ml. of concentrated hydrochloric acid and heat on a hot plate to dissolve, adding more acid if necessary. Cool, add 0.5 ml. of concentrated nitric acid, evaporate the solution to dryness, and bake carefully. Cool slightly, add 15 ml. of 72 per cent perchloric acid, and fume on a hot plate for 3-6 minutes, or until the solution is faintly yellow. Cool and use as sample, by the phosphovanadomlybdate method. Dissolve the sample in the 10 ml. of ammonium vanadate solution called for in the procedure.

Alternatively,[21] dissolve a 0.1-gram of finely ground sample in 3 ml. of concentrated hydrochloric acid. Dilute to about 10 ml. and filter. Add 1:1 ammonium hydroxide to approximate neutrality as shown by incipient precipitation of ferric ion. Add 2 ml. of 1:36 hydrochloric acid and use for development of the yellow phosphobolydate.

Manganese Ores.[22] Follow the methods for analysis of iron ores but use a smaller sample because the phosphorus content may be as high as 0.4 per cent. Reduce the solution with sodium sulfite before adding the reagents for the determination as molybdenum blue.[23]

Silicate Minerals.[24] Transfer a sample of 0.005-0.03 gram to a small platinum crucible. Add 2 drops of water to moisten the sample, then 0.25 ml. of concentrated nitric acid and 1 ml. of 48 per cent hydrofluoric acid. Cover the crucible and heat on a water bath for 10 minutes. At the end of that time rinse off the cover and evaporate the contents of the crucible to dryness. Add 0.5 ml. of concentrated nitric acid and evaporate to dryness. Repeat that step.

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[20] Hobart H. Willard and E. John Center, *Ind. Eng. Chem., Anal. Ed.* 13, 81-3 (1941); E. John Center and Hobart H. Willard, *ibid.* 14, 287-8 (1942).

[21] N. I. Pronenko and M. I. Kamyanyĭ, *Zavodskaya Lab.* 10, 423 (1941).

[22] V. A. Romashchenko, *ibid.* 11, No. 1, 104 (1945).

[23] E. I. Vovchenko and V. A. Romashchenko, *ibid.* 10, 643-4 (1941).

[24] I. P. Alimarin and A. Ya. Sheskol'skaya, *ibid.* 11, 259-62 (1945).

Dissolve the contents in 2 ml. of water and transfer to a beaker. Wash the crucible with 2 ml. of hot water. Heat the crucible with 1 ml. of 1:2 hydrochloric acid for a minute and add to the washings. Continue to wash the crucible with hot water but do not exceed a total volume of solution of 7 ml. Heat the covered beaker until solution of nitrates is complete. Add an aluminum shaving to reduce the ferric iron, rinse the cover glass, and filter if not completely clear.

If zirconium was present in the sample, it could be present as the phosphate in the insoluble fraction. Therefore if zirconium is present, ash the paper, and fuse the residue with 0.5 gram of sodium carbonate. Take up the melt with 3 ml. of water and filter. Wash the residue well and make the filtrate acid with 1:1 hydrochloric acid. Heat until evolution of carbon dioxide is complete. Combine this with the earlier solution of phosphate as sample and determine by reduction to molybdenum blue with stannous chloride.

Limestone. Perchloric acid is preferred over nitric acid to remove silica and prevent high results due to the formation of silicomolybdic acid.[25] Calcination helps to remove organic matter which would color the solution.

Ignite a sample of 5-0.5 grams correspondingly containing 0.002-0.18 per cent of phosphorus in a porcelain crucible for 30 minutes at 900° If the organic content is high, ignite first at 500° for 15 minutes, then follow by ignition at the higher temperature. Transfer to a beaker, add 20 ml. of water, and dissolve the calcium hydroxide in 72 per cent perchloric acid—18 ml. for 0.5 gram, 19 ml. for 1 gram, 20 ml. for 2 grams, and 25 ml. for 5 grams. Evaporate on a hot plate to fumes of perchloric acid. Cover with a watch glass and continue fuming for 5 minutes to dehydrate silica. Cool to 95°, filter, and use as aliquot for the determination of phosphorus, preferably by the phosphovanadomolybdate method.

Alternatively,[26] for limestone or dolomite moisten a sample of 0.005-0.03 gram in a beaker with 2 ml. of water and add 1:1 hydrochloric acid dropwise until evolution of carbon dioxide is completed. Add a drop of 4 per cent potassium permanganate solution and boil until this is decomposed. This will oxidize organic matter. Add a drop of saturated aqueous dinitrophenol and add 1:1 ammonium hydroxide until the solution is yellow Decolorize with a drop or two of 1:1 hydrochloric acid

[25] H. H Willard and E. J. Center, *Ind. Eng. Chem., Anal. Ed.* 10, 1 (1938); J. A Brabson, J H. Karchmer and M. S. Katz, *ibid.* 16, 553-4 (1944).

[26] I P. Alimarin and A. Ya. Sheskol'skaya, *Zavodskaya Lab.* 11, 141-5 (1945).

and add 0.8 ml. of 1:1 hydrochloric acid. This is a solution in form for determination of phosphate as molybdenum blue by stannous chloride reduction.

Orthophosphoric Acid in Phosphorous Acids. Other acids of phosphorus such as metaphosphoric and pyrophosphoric do not give the reaction. Suitably diluted they may therefore be used as sample, and their rate of hydration to orthophosphoric acid estimated. Reduction by aminonaphthol sulfonic acid is recommended.

Phosphorites.[27] To 5 grams of powdered sample add 50 ml. of 1:20 hydrochloric acid and shake mechanically for 30 minutes. Filter and dilute the filtrate to 250 ml. Withdraw 5 ml. and dilute to 250 ml. for the use of 5-ml. aliquots for reduction to molybdenum blue.

Commercial Pyrophosphate.[28] For commercial disodium hydrogen phosphate, dissolve 3 grams of sample in water at room temperature and dilute to 100 ml. For tetrasodium pyrophosphate dissolve a similar sample and add 1:1 hydrochloric acid dropwise until the solution is approximately neutral to litmus. Dilute this to 100 ml. For both, use as aliquot a portion containing 0.1-0.2 mg. of orthophosphate ion.

In either case modify the amount of 10 per cent ammonium molybdate in sulfuric acid added before reducing with hydroquinone. For up to 15 mg. of phosphorus pentoxide add 1 ml. of the 10 per cent solution in 1:17 sulfuric acid. For 15-30 mg. of phosphorus pentoxide use 2 ml. For 30-40 mg. use 3 ml., for 40-50 mg. use 4 ml. This is necessary because the pyrophosphate combines with some molybdate, and, although it gives no color reaction, the complex so formed is not reducible to molybdenum blue.

Alternatively,[29] dissolve 0.5 gram of finely ground sodium pyrophosphate containing 0.004-2 per cent of orthophosphate in 50 ml. of water and add a drop of phenolphthalein indicator solution. Add 1:6 sulfuric acid until the indicator is colorless and dilute to 100 ml. Transfer this to a separatory funnel and add 10 ml. of 1:2 nitric acid and 40 ml. of 10 per cent ammonium molybdate solution. Mix and add 5 ml. of butanol. Shake until it dissolves. To extract the yellow phosphomolybdate formed from the orthophosphoric acid, add 30 ml. of 1:3 butanol-

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[27] M. F. Zagorskii, *ibid.* 4, 1039-42 (1935).

[28] K. T. H. Farrer and S. J. Muir, *Australian Chem. Inst. J. & Proc.* 11, 222-6 (1944).

[29] R. V. Mervel, *Zavodskaya Lab.* 11, 135-7 (1945).

chloroform mixture. Invert the funnel about 20 times. Draw off the lower layer of extract. Repeat until the extract is colorless. Dilute the combined extracts to a known volume with butanol and compare with a standard similarly prepared (page 675). Each extraction will remove about 5 mg. of phosphorus as the complex. Since the reaction is conducted entirely in the cold, it is rapid enough to prevent hydration. Corresponding compounds with silicate or arsenate are not removed by the specified solvent. Therefore there may be a yellow color left in the solution being extracted.

Boiler Scale. The preparation of a sample was described under aluminum (page 242). Use 15 ml. of the solution so prepared and develop the color with ammonium molybdate and aminonaphtbol sulfonic acid.

Soils. *Total Phosphorus.*[30] Screen an air-dried rolled sample through 10-mesh. Discard the stems and stones so retained and grind the portion passing 10-mesh until it will pass 100-mesh. Digest 2-5 grams of sample with 30 ml. of 60 per cent perchloric acid under a hood until the solution is light and clear. Continue heating with occasional agitation for 20 minutes to ensure complete conversion of phosphorus to the inorganic form. Heavy white fumes appear, and the insoluble material appears like white sand. Use an additional 1-2 ml. of perchloric acid to wash down any black particles adhering to the sides. Cool and transfer to a volumetric flask, adding the washings of the residue to the contents of the flask. About 20 ml. of 60 per cent perchloric acid remain in this solution.

If arsenic is absent, dilute to an appropriate volume and mix thoroughly for use of aliquots for reduction to molybdenum blue with 1,2,4-aminonaphthol sulfonic acid.

If arsenate ions are present, transfer the aliquot to a 25-ml. volumetric flask and add enough 60 per cent perchloric acid to bring its concentration to 2.5 ml. Add 0.4 gram of solid sodium bisulfite, wash the neck of the flask, and dilute to 20 ml. Shake to dissolve the solid and allow to stand for 3 hours. This is ready for the development of color.

Total Phosphorus in Moor Soil.[31] Ash 50 grams of dry soil and take up the ash in a suitable volume of 1:9 hydrochloric acid. Add a few drops of concentrated nitric acid to assist the solubility. Evaporate to dryness and bake to dehydrate silica. Take up in 50 ml. of 1:9 hydro-

[30] Mildred S. Sherman, *Ind. Eng. Chem., Anal. Ed.* 14, 182 5 (1942).

[31] Th. Arnd and E. Leisen, *Bodenkunde u. Pflanzenernähr.* 30, 51-62 (1942).

chloric acid and filter the silica and other insolubles together. Dilute the filtrate to 500 ml. and evaporate a 100-ml. aliquot to dryness to remove the last of the silica. Heat on a steam bath with 5 ml. of 1:3 hydrochloric acid, then dilute nearly to 100 ml. and boil. Cool, dilute to 100 ml., and use an aliquot. The final solution must be no darker than a solution containing 10 ml. of 1:3 hydrochloric acid and 20 grams of ferric chloride hexahydrate per liter. The soil must not contain over 6 per cent of lime.

Iron Removal. Solutions of ash from soils with a high iron content are treated with 8-hydroxyquinoline to precipitate iron.[32] Prepare a buffer for pH 5.2 by mixing 2 volumes of saturated sodium acetate solution with 1 volume of 1:8 acetic acid. Prepare a solution of 8-hydroxyquinoline by dissolving 3 grams in a minimum amount of glacial acetic acid. Dilute nearly to 100 ml. and add 1:1 ammonium hydroxide until a slight precipitation occurs. Add 1:8 acetic acid dropwise until this just clears. Neutralize 25 ml. of the ash solution to litmus and add 10 ml. of the buffer. Warm to about 50° and add 0.4 ml. of the hydroxyquinoline reagent for each mg. of iron present. If no precipitate appears, add to a distinct coloration. If there is a distinct precipitate, filter and wash the filter with 3 per cent ammonium nitrate solution at about 65°. Let the filtrate cool. Extract the remaining solution 3 times with 10-ml. portions of ether so that the aqueous layer is colorless. Finally extract the aqueous layer with 10 ml. of benzene. Use the aqueous layer as sample or dilute to a known volume and use an aliquot. The ash may have part of the orthophosphate altered to pyrophosphate.[33] If the solution of ash is acidified and allowed to stand or if it is boiled, it is hydrated to orthophosphate again. Acidity in stannous chloride reduction will usually serve.

Organic Phosphorus.[34] Treat a 1-gram sample from which water-soluble phosphorus has been extracted, with 15 ml. of 30 per cent hydrogen peroxide that has been distilled under reduced pressure. For peat, use 25 ml. of the peroxide. Redistillation is necessary because of phosphate impurities in the usual 30 per cent hydrogen peroxide. Extract the residue with an appropriate volume of 1:175 sulfuric acid. Do not allow the soil to dry after treatment with hydrogen peroxide before

[32] E. Eisenberger and H. F. Przybylski, *ibid.* 17, 252-64 (1940).
[33] W. Bonewitz, *ibid.* 32, 106-19 (1943).
[34] S. R. Dickman and E. E. DeTurk, *Soil Sci.* 45, 29-39 (1938).

extraction or appreciable amounts of phosphorus will be fixed. Develop molybdenum blue in an aliquot of the extract with stannous chloride.

Soil Extract. *General.* Various extracts of soils have been proposed.[35] Water extraction gives that going into colloidal solution as humosilicophosphate. Extraction with 0.56 per cent calcium chloride solution saturated with carbon dioxide gives that present as calcium and magnesium phosphates. Extraction with a solution of 10 ml. of 0.1 *N* hydrochloric acid in 125 ml. of water gives that present as iron and aluminum phosphates. Each must be separately analyzed for phosphorus.

The silica content of soils generally leads to higher values,[36] and, if such is the case, correction should be made for its presence. Buffer effects due to salt concentrations can affect the intensity of molybdenum blue color developed.[37] Organic matter in soil extracts may interfere with reading the color but does not interfere with color development.[38]

If an appreciable amount of citric acid is used to extract phosphorus from the soil, it should be destroyed before proceeding with the determination.[39] In general stannous chloride reduction is preferred[40] but reduction with reduced molybdenum is also applicable.[41] The presence of magnesium or ammonium ion in moderate amounts does not adversely affect the molybdenum blue method.[42] A 10 per cent acetic acid extract of soil is suitable for development of molybdenum blue with stannous chloride.[43] For cytochemical investigations, where the phosphorus content is an indication of the nucleic acid content,[44] either aminonaphthol sulfonic acid or stannous chloride is a satisfactory reducing agent.

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[35] J. Clarens and J. Lacroix, *Bull. soc. chim.* 7, 377-82 (1940).

[36] F. Malychin, *Chem. Listy* 36, 2 6 (1942).

[37] John A. Schricker and Paul R. Dawson, *J. Assoc. Official Agr. Chem.* 22, 167-79 (1939).

[38] W. J. Dyer and C. L. Wrenshall, *Can. J. Research* 16B, No. 3, 97-108 (1938).

[39] J. Baeyens and D. Stenuit, *Agricultura* 39, 46-58 (1936).

[40] W. R. G. Atkins, *J. Agr. Sci.* 14, 192-7 (1924); D. Fehér, *Bodenkunde u. Pflanzenernahr* 1, 219-23 (1936); W. M. Holman and A. G. Pollard, *J. Soc. Chem. Ind.* 56, 339 43T (1937); S. R. Dickman and R. H. Bray, *Ind. Eng. Chem., Anal. Ed.* 12, 665 8 (1940); Carlos A. Fynn, *Rev. facultad agron. Univ. rep.* (Montevideo) No. 24, 33-40 (1941).

[41] J. Baeyens and D. Stenuit, *Agricultura* 39, 46 58 (1936).

[42] B R. Bertramson, *Soil Sci.* 53, 135 41 (1942).

[43] Carlos A. Fynn, *Rev. facultad. agron. Univ. rep.* (Montevideo) No. 24, 33 40 (1941)

[44] Bo Norberg. *Acta Physiol. Scand.* 5, *Suppl.* 14, 99 pp. (1942).

Hydrochloric Acid Extract.[45] As sample, use 0.5-1.0 gram of 60-mesh soil, depending on the amount of organic matter present. To the sample and to a standard of equal weight add 20 ml. of 1:100 hydrochloric acid and allow to stand for several minutes. If the soil is calcareous or contains undecomposed plant tissue, place on a steam bath for 5 minutes. Filter and wash with six 2-ml. portions of 1:100 hydrochloric acid or until calcium is no longer present in the washings. Dilute volumetrically to 200 ml. and reserve. This is the acid extract for later use.

Ammonium Hydroxide Extract. To extract alkali-soluble phosphorus transfer the acid-washed soil to a calibrated conical flask. Add 200-300 ml. of 1:30 ammonium hydroxide, stopper, and shake vigorously until the filter paper is shredded. Rinse the stopper and sides of the flask and dilute volumetrically with 1:30 ammonium hydroxide to 400 ml. Use rubber stoppers with Bunsen valves and digest in an oven at 89-91° for 16-18 hours. Cool the flask to room temperature in running water and add 5 grams of ammonium chloride. Adjust the volume to 400 ml., mix thoroughly, and allow to stand for a few minutes for the suspended matter to settle out. Decant through a filter, discarding the filtrate as long as any suspended material can be detected. The acid extract was diluted to half the volume of the alkaline extract.

Total Phosphorus. For the total phosphorus in the extract pipet one part of the acid extract and 2 parts of the alkaline extract. Usually 5 ml. and 10 ml. are suitable. Evaporate the aliquot to dryness with 1 ml. of 10 per cent magnesium nitrate and ignite in a muffle furnace at 600° until a completely white ash is obtained. Dissolve the residue in 4 ml. of 1:35 sulfuric acid and dilute to 20 ml. Filter and wash the filter. Dilute the filtrate to 40 ml., add 1 drop of 0.5 per cent *p*-nitrophenol indicator, and 1:1 ammonium hydroxide until the indicator just turns yellow. Add dropwise 1:35 sulfuric acid to the disappearance of color. Use this as sample for determination as molybdenum blue reduced with stannous chloride.

Inorganic Phosphorus. For this pipet out one part of the acid extract and 2 parts of the alkaline extract, usually 5 ml. and 10 ml. respectively. Add 4 ml. of 1:35 sulfuric acid and dilute to about 20 ml. Add 0.025 gram of carbon black. Swirl to mix the suspension thoroughly, filter, filling the funnel only half-full. Rinse the beaker and wash the filter with small portions of water. Determine as molybdenum blue by reduction with stannous chloride.

[45] R. W. Pearson, *Ind. Eng. Chem., Anal. Ed.* 12, 198-200 (1940).

Organic Phosphorus. Subtract inorganic phosphorus from total phosphorus.

Carbonic Acid Extract.[46] Bubble carbon dioxide through a 2 per cent suspension of the soil for 15 minutes. Filter and use an aliquot of the filtrate as sample for reduction to molybdenum blue by stannous chloride. Increasing temperature decreases the extraction. Within reason the soil:water ratio has no significant effect. Equilibrium is reached in about 10 minutes. Calcium carbonate reduces the solubility of the phosphate, calcium sulfate has a lesser but similar effect.

Sulfuric-acid-soluble Phosphorus in Soils.[47] Extract 2.5 grams of air-dry soil with 25 ml. of 0.05 N sulfuric acid to which sufficient sodium borate has been added to adjust the pH to 1.5 as measured by the glass electrode. Filter after 0.5 minute and determine the phosphorus in an aliquot, suitably 2 ml., by reduction to molybdenum blue with stannous chloride.

Hydrochloric Acid Extracts of Clay Soils.[48] Boil 20 grams of soil for 5 minutes with 70 ml. of concentrated hydrochloric acid and allow to digest on a water bath for 48 hours. Add water, filter, wash, and dilute to 250 ml. Treat 15 ml. of this extract with 0.5 ml. of 20 per cent sodium permanganate solution and heat on a sand bath for 15 minutes. The liquid should show no precipitated manganese dioxide. Cool, dilute to about 30 ml., and add 6 ml. of 10 per cent potassium ferrocyanide solution. This precipitates iron. Add 5 ml. of 10 per cent manganese sulfate solution and shake frequently. After 1 hour carefully add 1:1 ammonium hydroxide until the blue color just turns to purple. Add 3.5 ml. of 1:18 sulfuric acid and transfer to a 100-ml. volumetric flask. Dilute to volume and filter. Use a suitable aliquot of the filtrate for reduction to molybdenum blue by stannous chloride.

Citric Acid Extracts of Carbonate Soils.[49] Determine the amount of citric acid necessary to neutralize the sample according to the carbonate content. Add this quantity of 20 per cent citric acid and a 15-ml. excess to 5 grams of soil, dilute to make 50 ml., and shake for 1 hour. After 24

[46] Amar Nath Puri and A. G. Asghar, *Soil Sci.* **42,** 39-45 (1936).
[47] B. E. Beater, *Proc. 15th Ann. Conf. S. African Sugar Tech. Assoc.* **1941,** 113-20.
[48] R. G. Warren and A. J. Pugh, *J. Agr. Sci.* **20,** 532-40 (1930).
[49] A. V. Alekseeva, *Chemisation Socialistic Agr.* (U.S.S.R.) **1935,** No. 8, 41-3.

hours shake again for 1 hour. After 48 hours, filter and determine phosphorus by reduction to molybdenum blue with stannous chloride.

Citric Acid Extracts of Clay Soils. Shake 25 grams of soil for 24 hours in a 500-ml. bottle with 250 ml. of 1 per cent citric acid solution, with addition of excess citric acid to allow for calcium carbonate present. Filter and treat 75 ml. of the filtrate in a Kjeldahl flask with 10 ml. of concentrated hydrochloric acid and 12 ml. of 20 per cent sodium manganate solution. Let stand for one-half hour and heat until the precipitate of manganese dioxide disappears. Transfer to a 100-ml. flask and add 4 ml. of 10 per cent potassium ferrocyanide solution, drop by drop, with shaking. After 10 minutes carefully add 1:1 ammonium hydroxide until the blue color just turns to purple. Add 1.5 ml. of 1:17 sulfuric acid and dilute to volume. Filter and use an aliquot of the filtrate for reduction to molybdenum blue by stannous chloride.

Citric Acid Extracts of Soils Low in Calcium. Shake 10 grams of soil with 100 ml. of 1 per cent citric acid solution for 1 hour. A correction factor has to be applied to high-calcium soils. Repeat for 1 hour the next day. Filter or centrifuge and add 10 ml. of 2:3 sulfuric acid to 5 ml. of the soil solution. Oxidize the citric acid with 0.1 per cent potassium permanganate solution until a faint permanent pink color is obtained. Add 3 per cent hydrogen peroxide to decolorize the permanganate. Digest on a water bath for one-half hour and transfer to a 100-ml. flask. Add 4 drops of *o*-dinitrophenol as indicator and neutralize with 1:4 ammonium hydroxide. Cool and reduce the sample to molybdenum blue by stannous chloride.

Lactic Acid Extract of Soil.[50] Shake 5 grams of finely divided soil frequently for 2 hours with 250 ml. of a solution which is 0.02 N with calcium lactate and 0.01 N with hydrochloric acid, whose pH is 3.7. Filter and use a 25-ml. aliquot of clear extract for the determination of phosphorus, preferably by stannous chloride reduction to molybdenum blue. The solutions used in preparing the calibration curve must contain the same amounts of calcium lactate as the sample, since it affects the color development.[51]

Bone Meal.[52] To a 5-gram sample add 50 ml. of concentrated sul-

[50] H. Riehm, *Bodenkunde u. Pflanzenernahr.* 9-10, 30-50 (1938).
[51] E. G. Williams and A. B. Stewart, *J. Soc. Chem. Ind.* 60, 291-7 (1941).
[52] Kurt C. Scheel, *Z. anal. Chem.* 105, 256-69 (1936); *Ind. chimique* 23, 885-8 (1936).

furic acid and 5 ml. of concentrated nitric acid. Heat and, if necessary, add more nitric acid from time to time to complete the oxidation. Finally heat to strong sulfur trioxide fumes and let cool. Take up in water and dilute to 1 liter. Use a 1-ml. aliquot for reduction to molybdenum blue by aminonaphthol sulfonic acid or by monomethyl-*p*-aminophenol sulfate. It is desirable[53] to (1) buffer with sodium acetate at least equivalent to the amount of sulfuric acid in the final solution, (2) let the reduction proceed for 20 minutes, and (3) have only 0.5-1.0 mg. of phosphorus pentoxide per 100 ml. of final solution.

Water.[54] Ordinarily dilute with distilled water, or concentrate a suitable volume of sample according to the phosphate content, to 30 ml. and use as sample. Determine by the molybdenum blue method with stannous chloride reduction.[55] For waters not suitable for use directly,[56] for example boiler waters, add to a 100-ml. sample 0.2 ml. of 72 per cent perchloric acid and evaporate gently on a hot plate to fumes of perchloric acid. If the sample is hard water, additional acid may be necessary. If oxidation is slow, cover with a watch glass and digest until the coloration disappears. Let this solution cool and take up with water as the sample for reduction to molybdenum blue.

In water of varying salinity a salt effect is noticeable up to 0.5 per cent of sodium chloride, but this does not further increase appreciably up to 3 per cent.[57] Copper and iron to 4-5 times the amounts likely to be present do not interfere. By use of high acidity before adding the molybdate reagent, interference from silica is prevented. The amounts of the corrections depend both on the reagent used and the amount added. Hydrolytic products of complex molybdenum halides result in a yellow color from sea water which is noticeable in phosphate determinations, and correction factors should be applied.[58]

Determination of phosphates in underground spring waters, in waters of a turbid or colored nature, and in waters containing humic substances, must be preceded by ultrafiltration to remove suspended matter.[59] Following that, add 2 ml. of 1:1 sulfuric acid to 200 ml. of water followed

[53] P. Lederle, *Z. anal. Chem.* **121**, 403-11 (1941).

[54] L. S. Kahtaeva and B. E. Reznick, *Zavodskaya Lab.* **9**, 623-5 (1941).

[55] Waldemar Ohle, *Angew. Chem.* **51**, 906-11 (1938); N. T. Wilkinson, *J. Soc. Chem. Ind.* **57**, 292-5 (1938).

[56] Rex J. Robinson, *Ind. Eng. Chem., Anal. Ed.* **13**, 465-6 (1941).

[57] Charles E. Brambel and R. P. Cowles, *Science* **85**, 340-2 (1937).

[58] L. H. N. Cooper, *J. Marine Biol. Assoc. United Kingdom* **23**, 171-8 (1938).

[59] S. V. Bruevich and E. I. Pletnikova, *J. Applied Chem.* (U.S.S.R.) **9**, 925-31 (1936).

by immediate addition of 1 ml. of 10 per cent barium chloride solution to precipitate nearly all the colored matter. Let the suspension stand for 2-12 hours before filtration. If not ultrafiltered before addition of acid, the value will be high due to acid extraction of phosphorus from the suspended matter. Either phosphorus or silica alone can be determined by the molybdenum blue method or, by modification, either in the presence of the other.[60] In waters rich in silicic acid, extraction of the phosphomolybdic acid complex with acetoacetic ester, in which the complex is insoluble, is suitable.[61] Other solvents also perform this function. Phosphorus is estimated in boiler water containing as much as 300 ppm. of silica.[62] Interfering tannins in boiler waters are removed by coagulation with potassium nitrate, bleaching with potassium persulfate, or sorption by decolorizing carbon. The last is preferred.[63]

Alternatively,[64] treat a 10-ml. aliquot of sample with 0.25 ml. of concentrated nitric acid. Determine phosphate turbidimetrically by means of the strychnine-molybdate reagent.

Metaphosphate in Water.[65] To 100 ml. of filtered sample add 10 ml. of 1:8 sulfuric acid and a couple of pieces of broken Pyrex glass. Boil gently on a hot plate for 4 hours, adding distilled water to maintain the volume at 50-100 ml. Cool, dilute to volume, and use an aliquot for development of molybdenum blue with stannous chloride. Determine the orthophosphate in the original water, acidified but not boiled, and subtract this to give the corrected metaphosphate content.

Phosphatase. Extraction of the phosphatase may be carried out with alcohol-ether mixtures,[66] chloroform-water mixtures, saline solutions, and isotonic citrate solutions.[67] Phosphorus in these extracts is almost completely soluble in petroleum ether,[68] but precautions must be taken to control temperature and oxidation. The principle of measurement is to

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[60] T. S. Harrison and H. Storr, *J. Soc. Chem. Ind.* 63, 154-6 (1944).

[61] Karl Stoll, *Z. anal. Chem.* 112, 81-90 (1938).

[62] W. Demberg, *Die Chemie* 55, 318-19 (1942).

[63] L. Goldman and R. N. Love, *Power Plant Eng.* 50, No. 11, 76-9 (1946).

[64] F. Postic, J. Rabaté and J. Courtois, *J. pharm. chim.* 2, 122-5 (1942).

[65] Max Herzog, Wilkins Anderson Co. Waco Technical Bulletin, December, 1946.

[66] Miklós Berend and Mária Fischer, *Magyar Biol. Kutatóintézet Munkái* 10, 302-6 (1938).

[67] Hirosi Huzita, *J. Biochem.* (Japan) 30, 69-87 (1939).

[68] Evelyn B. Man, *J. Biol. Chem.* 117, 183-7 (1937).

incubate a substrate with the sample and a buffer for a known time at a known temperature. An ingredient of the substrate is then determined. Determination of several phosphatases such as in serum and corpuscles is given later.

Substances usually used as substrates are sodium glycerophosphate,[69] phenyl phosphate,[70] p-nitrophenyl phosphate,[71] and phosphotyrosine.[72] Incubation usually takes place at 37°-37.5°, but the pH of the optimum phosphatase activity depends on the time of reaction; at 21 hours, the optimum pH is 9, at 45 hours 8.7, and at 70 hours 8.65.[73] Tissue phosphatase acts preferentially on β-glycerophosphate without interference from magnesium ions if the pH is adjusted to 5. Action of blood phosphatase on α-glycerophosphate is affected by citrate ion.[74] Proper pasteurization of milk may also be checked, detecting as little as 0.2 per cent of raw milk.[75] Since the method as usually applied is but little more than qualitative, it is often incubated for 10 minutes at 37°. Cow's milk contains on the average 1.650 grams of phosphatide per liter.[76]

Among the buffers used are acetate barbital buffers and ammonium hydroxide-ammonium chloride buffers.[77] Comparison may be made directly or against a calibration curve.[78] The determination is influenced by oxalates, citrates,[79] silicates, and red corpuscular phosphatase. Hence nonhemolytic serum must be used. Other applications are to yeast,[80] salivary phosphatase,[81] serum phosphatase for the determina-

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[69] Aaron Bodansky, *J. Biol. Chem.* 101, 93-104 (1933); Morris Rhian, *Proc. S. Dakota Acad. Sci.* 19, 115-17 (1939); Geo. Y. Shinowara, Lois M. Jones and Harry L. Reinhart, *J. Biol. Chem.* 142, 921-33 (1942).

[70] Inger Buch and Holger Buch, *Acta Med. Scand.* 101, 211-36 (1939); Marco A. Mena Brito, *Prensa med. Mex.* 7, 127 (1942).

[71] Hirosi Huzita, *J. Biochem.* (Japan) 30, 69-87 (1939).

[72] Francis Binkley, R. E. Shank and Charles L. Hoagland, *J. Biol. Chem.* 156, 253 6 (1944).

[73] Erling Lundsteen and Emil Vermehren, *Compt. rend. trav. lab. Carlsberg. Sér. chim.* 21, 147-66 (1936).

[74] Eugen Bamann and Walter Salzer, *Biochem. Z.* 286, 143-6 (1936).

[75] H. Scharer, *J. Milk Tech.* 1, No. 5, 35-8 (1938); J. Wyllie, *Can. Pub. Health J.* 31, 147-55 (1940); *ibid.* 32, 122 8 (1941).

[76] L. Buruiana and A. Furtonesco, *Lait* 21, 8-14 (1941).

[77] D. M. Greenberg, S. P. Lucia and H. G. Weitzman, *J. Lab. Clin. Med.* 25, 634-41 (1940)

[78] Juan J. Lussich Siri, *Arch. uruguay. med., cirug. y especial.* (Montevideo) 24, 457 69 (1944).

[79] Eugen Bamann and Walter Salzer, *Biochem. Z.* 286, 143-6 (1936).

[80] James J. Rae and Edna V. Eastcott, *J. Biol. Chem.* 136, 443-7 (1940).

[81] James J. Rae, *J. Dental Research* 20, 453 6 (1941).

tion of rickets,[82] and lung fluids for determination of tuberculosis.[83]

A unit of alkaline phosphatase, pH ranging from 9.3 ± 0.15, is that amount of activity that will liberate 1 mg. of phosphorus as phosphate at 37° from sodium β-glycerophosphate in 30 minutes. A unit of acid phosphatase, pH 5.0 ± 0.15, is that amount of activity that liberates 1 mg. of phosphorus as phosphate at 37° in 1 hour.[84] In neither case must more than 10 per cent of the substrate be hydrolyzed.

With glycerophosphate the liberated phosphate is suitable for determination by conventional colorimetric methods. To extract,[85] let the finely comminuted material stand for 24 hours at room temperature in 5 times its weight of water saturated with chloroform. Centrifuge at 2400 rpm. or filter, and dilute to 500 times the original volume by adding a solution containing 0.739 per cent of magnesium sulfate and 10 ml. of a buffer selected according to conditions desired. To 9 ml. of the diluted sample add 1 ml. of 1 per cent dl-sodium glycerophosphate and 1 crystal of thymol. Allow to stand for 24 hours at 37°. Cool to room temperature and determine phosphate by reduction to molybdenum blue with stannous chloride.

Biological Materials, General.[86] *Total Phosphorus.* To a 10-gram sample of material, add 60 ml. of concentrated nitric acid and boil gently with stirring for 30-45 minutes, or until the gel-like particles formed disintegrate to a finer suspension. Cool somewhat, add 24 ml. of 70 per cent perchloric acid, and continue oxidation over a very low flame. Heat until colorless or a faint yellow. Evaporate to about 50 ml. and filter through an inorganic filter to remove silica. Dilute to 100 ml., boil for 30 minutes, cool, and make up to 250 ml. for use of aliquots. In analyzing colored solutions obtained from some samples, use as a reference standard a similar aliquot of sample treated exactly as the test solution but omitting the ammonium molybdate. The phosphovanadomolybdate method is applicable to biological materials.[87]

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[82] Ernst Muller, *Z. physiol. Chem.* 237, 35-9 (1935).

[83] Carlo Cattaneo and G. Scoz, *Klin. Wochschr.* 15, 1912-14 (1936); Carlo Cattaneo, G. Scoz and M. C. Gabbrielli, *ibid.* 16, 996-8 (1937).

[84] George V. Shinowara, Lois M. Jones, and Harry L. Reinhart, *J. Biol Chem.* 142, 921-33 (1942).

[85] Miklós Berend and Mária Fischer, *Magyar Biol. Kutatóintézet Munkái* 10, 302 6 (1938).

[86] Ruth Adele Koenig and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* 14, 155-6 (1942); Eugen Golenkin, *Bodenkunde u. Pflanzenernahr.* 36, 104-8 (1945).

[87] Paul Fleury and Maurice Leclerc, *Ann. pharm. franç.* 1, 101-4 (1943); *Bull. soc. chim. biol.* 25, 201-5 (1943).

Alternatively,[88] dissolve 0.04 gram of copper in 2 ml. of concentrated nitric acid. Boil this solution with an aliquot of sample containing 0.01-2.0 mg. of phosphorus. Heat to evaporate the acid and convert copper nitrate to copper oxide. Take up in 15 ml. of 1:4 hydrochloric acid, filter, and dilute to a suitable volume for use as sample. Reduction to molybdenum blue is desirable.[89]

Lipoid Phosphorus. Mix 1.0-1.5 grams of finely ground tissue with 3 grams of plaster of Paris and dry in a vacuum desiccator over sulfuric acid. Pulverize the mass with a little well-washed pulverized glass in a mortar. Filter on a Gooch crucible or fat-free paper. Wash the mortar with ether and pour the washings over the main precipitate.

Extract with anhydrous ether for 6 hours and absolute ethanol overnight. Repeat the next day. Extract the third day with absolute ethanol. Concentrate to a few ml. and dry in a vacuum desiccator over concentrated sulfuric acid. Dissolve the fatty matter with anhydrous chloroform and filter several times until clear. Wash the filter with hot chloroform. If desired, evaporate the extract and dry to give the weight of total extract and redissolve in chloroform. Dilute the solution to 25 ml., pipet out 2.5 ml. and evaporate to dryness. Ash by one of the usual methods and determine phosphorus in comparison with a standard chosen according to the nature of the sample.

Blood and Blood Fractions. *Total Phosphorus.* Ash 0.5 ml. of blood, plasma, or serum, or dilute to one-quarter strength with distilled water and ash a 2-ml. sample. Dissolve the ash in 1 ml. of 1:1 hydrochloric acid and dilute to a suitable volume. Develop the molybdenum blue color with stannous chloride and compare with 0.1 mg. of phosphorus.

Total Phosphorus in Corpuscles. Centrifuge blood and decant the plasma as quickly as possible Wash once with a volume of 0.9 per cent salt solution equal to the plasma, by shaking and centrifuging. If done quickly, there will be no significant loss of phosphorus by dialysis. Measure 1 ml. of corpuscles into a 25-ml. volumetric flask, rinse the pipet with water, and add the rinsings to the flask. Dilute to volume, mix, pipet 2 ml. as sample, and ash. Dissolve the ash in 1 ml. of 1:1 hydrochloric acid and dilute to a suitable volume. Develop the molybdenum

[88] Lucien Thivolle, *Bull. soc. chim. biol.* **17**, 1427-50 (1935).
[89] V. Zambotti, *Mikrochemie* **26**, 113-31 (1939).

blue color with stannous chloride[90] or aminonaphthol sulfonic acid.[91] The phosphovanadomolybdate method is also suitable.[92]

Inorganic Phosphorus in Blood, Serum, and Plasma.[93] Add 1.6 ml. of water to 0.1 ml. of sample in a centrifuge tube. Rinse the micropipet several times with the solution, add 0.3 ml. of 10 per cent trichloroacetic acid solution, mix, and centrifuge 3-5 minutes. Use 1 ml. of the centrifugate as aliquot and determine phosphorus, preferably by stannous chloride reduction to molybdenum blue, which is less duplicable but several times more sensitive than reduction with aminonaphthol sulfonic acid on these samples.[94] Errors may arise from the action of phosphatase, oxidizing properties of oxyhemoglobin, or hydrolyzing action of the acid.[95]

Inorganic Phosphorus in Corpuscles. Measure 5 ml. of corpuscles, previously washed with 0.9 per cent saline solution, into a 10-ml. volumetric flask and rinse any residue in with warm water. Dilute to volume, mix well, and let stand for 10 minutes with occasional shaking to allow the corpuscles to lake. Run 5 ml. slowly with mixing into 18 ml. of 10 per cent trichloroacetic acid solution in a 25-ml. volumetric flask. Mix well and dilute to volume with 10 per cent trichloroacetic acid solution. Filter a portion, develop the color in 5 ml. of filtrate and compare with a standard containing 0.05 mg. of phosphorus or read photometrically. Add 4 ml. of 10 per cent trichloroacetic acid to the standard to make conditions comparable.

Lipoid Phosphorus in Blood, Plasma, Serum, or Bile. The lipoid phosphorus is the lecithin fraction. Pipet 3 ml. of whole blood, plasma, or serum dropwise into 35 ml. of a mixture of 1:3 redistilled ethyl ether and redistilled 95 per cent ethanol, with swirling to eliminate lumping. Place in a boiling water bath to boil the mixture, shaking to avoid super-

[90] Pellervo Saarinen, *Maataloustieteellinen Aikakauskirja* **10**, 128-39 (1938).

[91] Henry L. Brose and Ernest B. Jones, *Nature* **138**, 644 (1936); Jonas Kamlet, *J. Lab. Clin. Med.* **22**, 966-7 (1937); Kurt C. Scheel, *Z. anal. Chem.* **105**, 256-69 (1939); K. Kropp, *Z. Kinderheilk.* **61**, 601-12 (1940).

[92] Enrique Alfonso Gordo, *Med. espan.* **11**, 668-72 (1944).

[93] L. A. Rutkovskii, *Lab. Prakt.* (U.S.S.R.) **15**, No. 5, 18-21 (1940); K. Kropp, *Z. Kinderheilk.* **61**, 601-12 (1940); cf. Basil Soyenkoff, *J. Biol. Chem.* **168**, 447-57 (1947).

[94] Joseph M. Looney and Cora G. Dyer, *J. Lab. Clin. Med.* **27**, 554-6 (1942).

[95] Pellervo Saarinen, *Maataloustieteellinen Aikakauskirja* **10**, 128-39 (1938).

heating. Cool to room temperature, dilute to 50-ml. with the alcohol-ether mixture, mix well, and filter. Protect the filtrate from evaporation.

Transfer 2 ml. of extract to a Pyrex test tube. Add a glass bead and place in a boiling water bath to evaporate the extract to dryness. Add 0.5 ml. of 1:2.5 sulfuric acid and place over a microburner at an angle, adding 1-2 drops of 30 per cent hydrogen peroxide if necessary to clear the solution. Pass the tube over an open flame to drive off fumes. Cool, add 2 ml. of water, and heat to boiling to convert meta- and pyrophosphate ions to orthophosphate. Use this as sample for determination by stannous chloride reduction to molybdenum blue.

Lipoid Phosphorus in Corpuscles. To hemolyze, dilute with an equal volume of warm water and let stand for 10 minutes. Extract the lipoids from 6 ml. of this dilution as for whole blood except that the flask is shaken occasionally for 30 minutes to avoid the tendency of the precipitate to aggregate before heating. Use 10 ml. of extract, an aliquot corresponding to 0.3 ml. of corpuscles, and compare with a standard containing 0.01 mg. of phosphorus.

Hydrolyzable Organic Phosphorus in Blood. Blood contains a hydrolyzable organic phosphate which is largely precipitated in obtaining the plasma. To 5 ml. of clear plasma add reagents as usual for the hydroquinone method, omitting the sulfite. Mix, stopper loosely, and heat in boiling water for 15 minutes. Cool and develop molybdenum blue. Dilute 2 ml. of whole blood filtrate with 3 ml. of water and determine in the same way except that the period of heating is one hour. Compare each with a 0.03 mg. standard. The difference, after allowing for the aliquots taken, represents hydrolyzable organic phosphorus.

Phosphatase in Corpuscles.[96] Dilute 1 volume of 10 per cent sodium citrate solution with 9 volumes of isotonic salt solution. To 5 ml. of this add 0.1 ml. of blood. Centrifuge to separate the corpuscles without hemolysis. Wash the corpuscles with 10 ml. of physiological saline solution. Suspend the corpuscles in 20 ml. of 3.5 per cent sodium citrate solution, checked as having a pH of 6.0. Warm 5 ml. of this suspension to 37° and add 5 ml of fresh 0.233 per cent solution of disodium-p-nitrophenyl phosphate. After 1 hour at 37° add 10 ml. of 10 per cent trichloroacetic acid. Filter and use 5 ml. of the deproteinized filtrate as sample for determination.

[96] Hirosi Huzita, *J. Biochem.* (Japan) 30, 69 87 (1939).

Serum Phosphatase.[97] To prepare the substrate, introduce successively into a 100-ml. flask 3 ml. of petroleum ether boiling at 20-40°, 80 ml. of water, 0.5 gram of sodium *dl*-glycerophosphate, 0.424 gram of sodium diethyl barbiturate, and water to dilute the aqueous layer to volume. The petroleum ether floats on the surface and protects against oxidation. Store in a refrigerator, with additional petroleum ether added if necessary. Heat 10 ml. of substrate at 37° in a tube and add 1 ml. of serum. Swirl to mix and heat for 1 hour. Cool in ice water and add 9 ml. of 10 per cent trichloroacetic acid solution. Mix, let stand for a few minutes, and filter. Prepare a control by the same procedure but do not heat. Use an aliquot of each for determination of phosphate as molybdenum blue by reduction with stannous chloride or diaminophenol.[98] Alternatively, the liberated phenol can be determined.

Urine. *Total Phosphorus.* Ash a 1-ml. sample and dissolve the ash in 1 ml. of 1:1 hydrochloric acid. Use this as sample for reduction to molybdenum blue by stannous chloride.

Inorganic Phosphorus. Often 1-2 ml. containing 0.2-0.5 mg. of phosphorus may be diluted and used directly. If organic matter must be removed,[99] mix 10 ml. of urine with 10 ml. of 33 per cent trichloroacetic acid and shake with 1 gram of animal charcoal. Filter for use of aliquots and reduce to molybdenum blue.

Organic Phosphorus.[100] This cannot be accurately determined by difference because it is relatively small. Measure 20 ml. of urine into a 25-ml. v 'umetric flask. Render faintly alkaline with powdered barium hydroxide, thus precipitating the inorganic phosphorus, dilute to volume, and filter. To remove excess barium, measure 20 ml. of filtrate into a 25-ml. volumetric flask, render very faintly acid with sulfuric acid, dilute to volume, and filter. Avoid the use of excess sulfuric acid. One ml. of this filtrate is equivalent to 0.64 ml. of the original urine.

Ash 10 ml. of the above filtrate and determine phosphorus by comparison with a standard equivalent to 0.025 mg. of phosphorus. If this standard is not dark enough, it is better to repeat the digestion with a smaller amount of the filtrate than to increase the strength of the standard.

97 Aaron Bodansky, *J. Biol. Chem.* **99**, 197-206 (1932); *ibid.* **101**, 93-104 (1933)
98 Ernst Müller, *Z. physiol. Chem.* **237**, 35-9 (1935).
99 G. Barac, *Bull. soc. chim. biol.* **20**, 1278-81 (1938); *ibid.* **21**, 139-42 (1939).
100 Carl Urbach, *Biochem. Z.* **239**, 182-5 (1931).

Cerebrospinal Fluid.[101] *Total Phosphorus.* Digest 1 ml. of fluid, 2 ml. of water, and 0.5 ml. of 14 per cent trichloroacetic acid with 3 drops of concentrated sulfuric acid for 3 minutes on a boiling water bath. Transfer to a 5-ml. centrifuge tube, centrifuge for 20 minutes at 2000 rpm., and use 2-ml. portions of the supernatant liquid as the sample for reduction to molybdenum blue with stannous chloride.

Inorganic Phosphorus. Transfer a 1.5-ml. sample to a 5-ml. centrifuge tube, add 1.5 ml. of 14 per cent trichloroacetic acid, place in a boiling water bath for 3 minutes, and centrifuge. Use 2-ml. portions of the supernatant liquid as sample for reduction to molybdenum blue with stannous chloride.

Acid-soluble Phosphorus. Transfer 2 ml. of the de roteinized filtrate for inorganic phosphorus to a combustion flask. Digest with 3 drops of concentrated sulfuric acid, then with 3 drops of hydrogen peroxide. Make the solution alkaline to phenolphthalein with 40 per cent sodium hydroxide and then neutralize with 1:35 sulfuric acid. Add 1 ml. of 9.3:100 sulfuric acid and use for reduction to molybdenum b'ue with stannous chloride.

Lipoid Phosphorus. Transfer a 5-ml. sample to a separatory funnel and shake with 4 ml. of 95 per cent redistilled ethanol, then with 5 ml. of redistilled ethyl ether, and filter the combined extracts. Repeat the extraction with 5 ml. of ethyl ether, evaporate the extracts to 2 ml., and transfer to a combustion flask. Proceed as outlined for acid-soluble phosphorus, beginning with "Digest with 3 drops of concentrated sulfuric acid . . ."

Acid Phosphatase in the Prostate.[102] High phosphatase may indicate cancer of the prostate. Prepare a solution of 1.09 grams of phenyl sodium phosphate in 1 liter of a buffer solution containing 900 ml. of 0.7 per cent sodium citrate and 100 ml. of 1:100 hydrochloric acid. Mix 10 ml. of this reagent with 0.5 ml. of prostate serum and incubate for an hour at 37.5°. This is ready for estimation of the phenol tyrosine liberated as a measure of the phosphatide content.

Prepare a buffer solution of 900 ml. of 7 per cent sodium citrate solution and 100 ml. of 1:120 hydrochloric acid. Dissolve 1.09 gram of

[101] Caspar Tropp, Otto Seuberling and Bruno Eckardt, *Biochem. Z.* 290, 320-6 (1937).

[102] Marco A. Mena Brito, *Prensa méd. Mex.* 7, 127 (1942).

phenyl disodium phosphate in this. Mix 10 ml. of this with 0.5 ml. of sample solution and keep at 37.5° for 1 hour. Add 4 ml. of a 0.1 per cent solution of phenol in 1:120 hydrochloric acid and filter. Repeat without the heating as a control. To each add 2.5 ml. of 20 per cent sodium carbonate solution and use as a sample for determination of phosphate.

Salivary Phosphatase.[103] Follow the technic for preparation of the substrate and its activity shown for serum (page 652).

Milk. Add 2 ml. of milk to 5 ml. of water in a graduated cylinder. Add 2 ml. of 20 per cent trichloroacetic acid, and dilute to 10 ml. Mix and filter to obtain a clear solution. Use 5 ml. of filtrate, equivalent to 1 ml. of milk, as sample.

Cane Juice. Evaporate and ignite 20 ml. of cane juice with 4 drops of 10 per cent calcium acetate solution. Extract the ash with 10 ml. of 1:9 sulfuric acid and filter into a 100-ml. flask. Wash the residue and filter until free of acid, and dilute to volume. Use a suitable aliquot as sample for reduction to molybdenum blue with stannous chloride.

Sugar. Mix 5 grams of sugar in a platinum dish with 0.2 gram of anhydrous sodium and potassium carbonates. Char carefully over a free flame and finally incinerate in a muffle furnace below a temperature at which the ash will fuse. Cool and dissolve the ash in 1 ml. of 1:1 nitric acid. Render the silica insoluble by evaporating to dryness on the steam bath. Add 0.5 ml. of concentrated nitric acid and again evaporate to dryness on the steam bath. Take up with 5 ml. of water and filter at once to remove silica, catching the filtrate in a 100-ml. Nessler tube. Wash the filter until free of acid and use this solution as sample for reduction to molybdenum blue with stannous chloride.

Plant Material. *Total Phosphorus.*[104] To 1-2 grams of sample in a small Sillimanite crucible, add 1 ml. of 16 per cent magnesium nitrate solution in 1:1 nitric acid. Heat on a steam bath and, after a few minutes, add cautiously a few drops of concentrated hydrochloric acid, taking care that formation of gas bubbles does not push portions of sam-

[103] James L. Rae, *J. Dental Research* 20, 453-6 (1941).

[104] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Fifth Edition. pp. 127-8. Association of Official Agricultural Chemists, Washington, D. C. (1946); cf. H. W. Gerritz, *J. Assoc. Official Agr. Chem.* 22, 131-7 (1939); *ibid.* 23, 321-54 (1940); *ibid.* 24, 393-7 (1941).

ple over the edge of the crucible. Make further additions of a few drops of concentrated hydrochloric acid while the sample is on the bath so that as it approaches dryness there is a tendency for it to char. If the contents of the crucible become so viscous that no further drying can be obtained on the water bath, complete drying on a hot plate. Cover the crucible, transfer to a cold muffle, and ignite at a dull red heat for 6 hours, or until an even gray ash is obtained. It may be necessary to cool the crucible, dissolve the ash in a little water or alcoholic glycerol, evaporate to dryness, and return uncovered to the muffle for 4-5 hours longer. Cool, take up with a small volume of 1:4 hydrochloric acid, and transfer to a beaker. Add 5 ml. of concentrated hydrochloric acid and evaporate to dryness on a steam hatb to dehydrate silica. Moisten the residue with 2 ml. of concentrated hydrochloric acid, add about 50 ml. of water, and heat for a few minutes on the water bath. Transfer to a 100-ml. volumetric flask, cool, and dilute to volume. Filter, discarding the first portion of the filtrate. Use the molyhdenum blue method with reduction by hydroquinone.

Detailed methods for wet ashing of plant material were given (page 550) in tho potassium chapter. Use an aliquot as an alternative sample for determination of phosphorus. An aliquot of solution A prepared under determination of lead (page 30) is also suitable for estimation of phosphorus as orthophosphate. An extract prepared by treatment of the ash in platinum with 1 ml. of 1:4 hydrochloric acid if diluted to 100 ml. does not contain enough chloride to interfere with reduction [105] to molybdenum blue by aminonaphthol sulfonic acid [106] or monomethyl-*p*-aminophenol sulfate.[107]

Various other methods of preparation may be used. One is given for determination of magnesium (page 618). Use 5 ml. of solution A for development with ammonium molybdate and 1,2,4-aminonaphthol sulfonic acid.

As another method,[108] ash 1-2 grams of dried sample in a platinum crucible in a muffle furnace. Cool, add 10 ml. of 1:1.4 hydrochloric acid, and warm on a hot plate to 90°. Transfer to a 100-ml. volumetric flask, dilute to volume, and filter for use of aliquots.

Alternatively,[109] weigh 1-gram samples of material ground to 60-

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[105] F. W. Muller, *Bodenkunde u. Pflanzenernahr* 4, 13-16 (1937); L. Schmidt, *ibid.* 4, 10-13 (1937); *Forschungsdienst* 3, 596-600 (1937).

[106] Benjamin Wolf, *Ind. Eng. Chem., Anal. Ed.* 16, 121-3 (1944).

[107] J. Schnell and R. Frisch, *Bodenkunde u. Pflanzenernähr* 21/22, 341-3 (1940).

[108] C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* 14, 762-4 (1942).

[109] W. A. Pons, Jr. and John D. Guthrie, *ibid.* 18, 184-6 (1946).

mesh or finer and mix with 50 ml. of 12 per cent trichloroacetic acid. Shake mechanically for 1 honr and filter, discarding the first portion of the filtrate. Make up to a known volume and use an aliquot by the extraction method with stannous chloride rednction.

For grasses,[110] mix 1 gram of dried finely gronnd material with 50 ml. of a 1 per cent solution of magnesium nitrate, evaporate to dryness, and ignite. Dissolve the residue in 1:2.5 sulfuric acid and dilute to a known volume for the use of aliquots. Obtain molybdenum blue by reduction with aminonaphthol snlfonic acid. A sample prepared for determination of potassinm in fruit and fruit products (page 552) is suitable for determination of phosphorus as molybdenum blue in an aliquot. The phosphovanadomolyhdate method is also suitable[111] for vinegar, dried grass, kelp concentrates, tomato, spinach, liver, milk powder, haking powder, fertilizer, etc.

Phosphatides. Extract a sample of 1-2 grams, dried at not over 60°, for 5 hours with 50 ml. of 95 per cent ethanol. Distill the ethanol and treat tbe residue witb 1 ml. of concentrated snlfnric acid and an equal amount of concentrated nitric acid. Heat until decomposition is complete and let cool. Add 2 ml. of water, heat to hydrolyze nitrosyl sulfuric acid, and evaporate tbe nitric acid. Let cool and take up with water. Dilute to 100 ml. and use an aliquot for molybdenum blue by stannous chloride reduction.

Inorganic Phosphorus. Dry the extracted sample at 50° and shake for 1 hour with 120 ml. of 1:100 hydrochloric acid. Dilute to 200 ml. and filter. Use an aliquot as before. Inorganic phosphorus is also determined by extraction with dilute sulfuric acid[112] or etbanol acidified with hydrochloric acid.[113] These methods often extract sufficient protein to form a precipitate on the addition of molyhdate reagent. The addition of trichloroacetic acid[114] at 0.37-1 N eliminates the formation of such a precipitate. If the developed plant extract is cloudy, eliminate excess molybdic acid with oxalic acid and extract the blue color with isobutyl

110 A. W. Greenhill and N. Pollard, *J. Soc. Chem. Ind.* 54, 404-6T (1935).

111 Ruth Adele Koenig and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* 14, 155-6 (1942).

112 B. R. Bertramson, *Plant Physiol.* 17, 447-54 (1942).

113 R. C. Collison, *J. Ind. Eng. Chem.* 4, 606-9 (1912); *J. Biol. Chem.* 12, 65-72 (1912).

114 W. A. Pons, Jr. and John D. Guthrie, *Ind. Eng. Chem., Anal. Ed.* 18, 184-6 (1946).

alcohol.[115] Heat treatment of the plant extract may lead to the formation of dehydrated phosphoric acid and to low results.[116] By stannous chloride reduction a suitable acidity for reversion is supplied without undue difficulty.

Organic-acid-soluble Phosphorus. Evaporate 50 ml. of the extract obtained with hydrochloric acid, after adding 2 ml. of concentrated sulfuric acid and 2 ml. of concentrated nitric acid. When decomposition is complete, cool, take up in water, dilute to a known volume, and use an aliquot. Subtract from the phosphate so found, that present as inorganic phosphate.

Nucleo-proteides.[117] Subtract from the total content of phosphorus in the plant the amount of phosphorus in the form of phosphatides and acid-soluble phosphorus.

Peanut Meal.[118] Extract a 1-gram sample with petroleum ether to remove oils and proceed as for plant materials.

Proteins and Phospholipids.[119] To 30-40 mg. of protein or to 15-20 mg. of phospholipid in a micro-Kjeldahl flask, add 10 ml. of 1:2 5 sulfuric acid. Heat over an open flame to remove water and to char the sample. Cool, add a drop of 30 per cent hydrogen peroxide, and heat slowly with agitation to clarify the solution. Cool and repeat the addition of peroxide until the solution is clear. Cool, add 3-5 ml. of water, and heat to boiling to convert pyrophosphate to orthophosphate. Cool, dilute with water to a known volume, and transfer an aliquot containing 0.05-0.5 mg. of phosphorus to a 25-ml. volumetric flask. Add sufficient 1:2.5 sulfuric acid to make the acid concentration approximately 1:18. Determine by stannous chloride reduction to molybdenum blue.

Miscellaneous Organic Materials.[120] A rapid digestion method includes the molybdate in the mixture. As digestion mixture dissolve 30

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[115] Russell J. L. Allen, *Biochem. J.* 34, 858-63 (1940).

[116] W. Bonewitz, *Bodenkunde u. Pflanzenernähr.* 32, 106-19 (1943).

[117] A. V. Sokolov, *Chemisation Socialistic Agr.* (U.S.S.R.) 1940, No. 10, 36-8.

[118] W. A. Pons, Jr. and John D. Guthrie, *Ind. Eng. Chem., Anal. Ed.* 18, 184-6 (1946).

[119] T. D. Fontaine, *Ind. Eng. Chem., Anal. Ed* 14, 77-8 (1942); *cf.* Hans Wuhrmann and Otto Högl, *Mitt. Lebensm. Hyg.* 35, 273-89 (1944).

[120] Donald W. Bolin and Olof E. Stamberg, *Ind. Eng. Chem., Anal. Ed.* 16, 345 (1944).

grams of sodium molybdate in 150 ml. of water and slowly add 150 ml. of concentrated sulfuric acid. Let cool and add 200 ml. of 72 per cent perchloric acid.

To a 0.5-gram sample in a 100-ml. Kjeldahl flask add 5 ml. of the digestion mixture and a few glass beads to prevent bumping. Heat over a micro burner. Oxidation begins in 1-2 minutes. Remove the burner and allow oxidation to continue. Wash down any adhering particles on the sides of the flask, add 2 ml. of 72 per cent perchloric acid, replace the burner, and heat until digestion is complete, usually within 3-4 minutes. Dilute the clear solution to 100 ml. Filter or allow to stand to permit silica to settle out. Use an aliquot for determination of phosphorus, adjusting the perchloric acid content if necessary and allowing for the molybdate already added.

Mineral Oils.[121] Transfer a 1-gram sample containing 0.005-0.04 per cent of phosphorus to a porcelain crucible and cover with an approximately equal weigh of zinc oxide. Place in a tilted position over a luminous flame to burn the oil slowly, then ignite until the crucible is entirely free of carbon. Cool somewhat, place in a beaker, and empty the contents by tapping the crucible against the side of the beaker. Cover with water and add 10 ml. of 1:2 sulfuric acid. Heat on a hot plate, stirring occasionally to effect solution. Remove the crucible with washing. Transfer the solution to a 250-ml. volumetric flask and dilute to volume. To a 25-ml. aliquot diluted to 50 ml., add dropwise a 20 per cent sodium carbonate solution until the solution is turbid due to the formation of zinc hydroxide. Add with swirling 1:2 sulfuric acid dropwise until the solution clears on the addition of the last drop. Alternatively, 2,4-dinitrophenol may be used as indicator, changing from yellow to colorless. All the phosphorus is now present as orthophosphate. Use an aliquot for determination by stannous chloride reduction to molybdenum blue.

Calcium carbonate can be used[122] instead of zinc oxide, but in that case subsequent use of sulfuric acid must be avoided. Alkali carbonates require longer ignition and are more difficult to remove from the crucible. Magnesium nitrate is unsuitable because the magnesium ion affects the color and must therefore be present in standardized amount in sample and standard. Nitrates must be eliminated by subsequent decomposition during ignition. Zinc ion has no more than a negligible effect on

[121] Paul Goodloe, *ibid.* 9, 527-9 (1937).

[122] B. W. Howk and E. E. DeTurk, *ibid.* 4, 111-12 (1932).

the color developed. Zinc peroxide formed on ignition is active in the oxidation.

Separation of Arsenate, Phosphate, and Silicate. A method of separation is given under arsenic (page 188) by which the corresponding molybdate complexes are obtained in non-aqueous solution. They need then only be read against a corresponding standard (page 675).

Precipitation of Phosphate by Magnesia Mixture. Phosphate ion precipitated as magnesium ammonium phosphate by addition of an excess of magnesia mixture may be filtered, dissolved in nitric acid, and developed colorimetrically. The advantages are that all silica is removed from the solution, thus preventing errors due to silicomolybdate, and that the organic matter is absent from the final solution.

Neutralize and adjust the volume of a sample containing 0.5-5 mg. of phosphorus pentoxide to about 50 ml. Add a drop of concentrated ammonium hydroxide and 2-3 drops of a saturated solution of ammonium oxalate to the sample and evaporate to dryness on the water bath. Prepare magnesia mixture from 13 grams of crystallized magnesium chloride, 20 grams of ammonium chloride, and 50 ml. of concentrated ammonium hydroxide in 900 ml. of water. Dilute to 1 liter. Add 1 ml. of this solution to the dried precipitate and mix with a glass rod. After standing for 2 hours, wash the precipitate on the filter with 5 ml. of 1:9 ammonium hydroxide. Wash the dish and filter with further successive portions until the volume of the filtrate reaches approximately 50 ml. Wash the dish and filter with 5 ml. of water and discard the washings. This eliminates a trace of silica present in ammonium hydroxide. Place a clean beaker to catch the solution of the precipitate. Add 5 ml. of 1:5 nitric acid to dissolve any precipitate remaining on the dish and carefully dissolve the precipitate on the filter with this same acid. Wash the dish and filter with water and use as sample, or more commonly dilute to a known volume and use an aliquot.

Standard

Dissolve 0.4263 gram of ammonium monohydrogen phosphate, $(NH_4)_2HPO_4$, in water and make up to 1 liter. Each ml. will contain 0.1 mg. of phosphorus Alternatively, recrystallize monobasic potassium phosphate 3 times from water, dry at 110°, and keep in a desiccator over concentrated sulfuric acid. Dissolve 0 4393 gram of dry salt in 300 ml. of water and 200 ml. of 1:35 sulfuric acid. Add a few drops of 2 per

cent potassium permanganate as preservative and dilute to 1 liter. Each ml. will contain 0.1 mg. of phosphorus per ml. To use disodium hydrogen phosphate, Na_2HPO_4, dissolve 0.2292 gram per liter of 1:50 nitric acid. Each ml. is equivalent to 0.05 mg. of phosphorus. Store phosphate standards in old volumetric flasks.

Orthophosphate as Molybdenum Blue

Very small amounts of phosphorus may be determined by reduction of the yellow phosphomolybdate complex to molybdenum blue.[123] Although not one of the newer methods for determination of phosphorus, it is still the outstanding one. An enormous amount of investigation has been devoted to the molybdenum blue reaction. Apparently the phosphomolybdic acid complex is more readily reduced than the molybdate, and therefore a proportional yield of molybdenum blue is obtained. A corresponding formation of silicomolybdate, arsenomolybdate, germanomolybdate, etc., explains other cases of formation of molybdenum blue.

Molybdenum blue derived from phosphate not only serves as the basis for the colorimetric determination of phosphorus, but also for various indirect determinations where the test substance has been isolated as orthophosphate. Magnesium separated as magnesium ammonium orthophosphate is an example. Care must be taken to so control conditions as to avoid reduction of the excess molybdate reagent to molybdenum blue.

Although conditions can be controlled to obtain reaction of the phosphate without arsenate or silicate, it is simpler and safer to have silicate and arsenate absent. Suitable methods have been given for their removal from many kinds of samples. Interference by arsenate is avoidable by addition of thiourea which reduces it to arsenite.[124] There are no specific reducing agents, almost any one producing the blue color in time. Solutions of the complex show some increase in the intensity of color on standing. The presence of ferrous ion may lead to erroneous results by reduction of the molybdic acid.[125]

Low concentrations of reactants apparently yield a true solution of the molybdenum blue complex, but there is reason to believe that the system is actually colloidal.[126] In measuring the intensity of a reduced molybdenum blue solution, the color once developed can rarely be diluted,

[123] Richard D. Bell and Edward A. Doisy, *J. Biol. Chem.* 44, 55-67 (1920).
[124] Waldemar Ohle, *Angew. Chem.* 51, 906-11 (1938).
[125] A. Malkov, *J. Applied Chem.* (U.S.S.R.) 19, 577-9 (1946).
[126] Rudolf Rinne, *Z. anal. Chem.* 113, 241-7 (1938).

since the intensity depends on the pH, the molyhdate-acid ratio, the amount of molyhdate, the intensity of light, and the presence of other ions.[127] An anion other than that of the acid used in the reagent influences the color intensity, as for example when chloride ions are present with a sulfuric acid reagent.[128] Increases in temperature increase the rate of development of color. If sufficient reagent and reductant are present to hring ahout complete reaction, any excess does not greatly affect the color. With a given concentration of molyhdate a minimum concentration of acid is required to prevent color development in the ahsence of phosphate. Just ahove this critical concentration, there is a limited range in which the intensity of color is proportional to the phosphate content almost independently of the acidity. Further increases in acidity result in a decrease in color intensity.

At higher acid concentrations, increasing the anion, such as chloride ion, does not have a great effect on the results.[129] The rate of fading increases as the phosphate-ion concentration increases.[130] Generally, if a sample is dissolved in nitric acid and then evaporated to fumes with perchloric acid, an effort should he made to remove as much as possihla of the nitric acid with a minimum loss of the perchloric acid.

To control the acidity, buffer solutions are generally used. These usually change the hlue-green color which first develops to the hlue molyhdenum-complex color. The most common are sodium sulfite, sodium succinate, or sodium acetate. By using a sodium succinate huffer, the stability may he increased when the final pH of the colored system is 4.0-4.7.[131] The maximum color stahility is developed at pH 2.1-2.7 for solutions standing 1 hour. Additional succinate buffer causes the color to fade. When a 20 per cent solution of sodium sulfite is suhstituted for sodium succinate, maximum color development occurs, hut the color tends to fade. Solutions without sodium sulfite are greenish blue, and their transmittance curves show a maximum at 465 mμ. The addition of the sulfite produces an intense blue color, and the maximum transmittance shifts to 445 mμ. Increasing the sulfite results in more intense color, increased pH, and more stahle solutions. Different reducing agents result in different transmittance curves, the deviation increasing in general with increase in wave length. Solutions whose pH ranges from

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[127] Hobart H. Willard and E. John Center, *Ind. Eng. Chem., Anal. Ed.* 13, 81-3 (1941); J. T. Woods and M. G. Mellon, *ibid.* 13, 760-4 (1941).

[128] S. R. Dickman and R. H. Bray, *ibid.* 12, 665-8 (1940).

[129] O. Hoffman, *Bodenkunde u. Pflanzenernähr.* 4, 16-18 (1937).

[130] W. J. Dyer and C. L. Wrenshall, *Can. J. Research* 16B, No. 3, 97-108 (1938).

[131] L. S. Stoloff, *Ind. Chem., Anal. Ed.* 14, 636-7 (1942).

2.3-5.2 are stable for 1.5 hours; from 2.3-4.7, they are stable for 4.5 hours. The color intensity after development is constant for solutions whose pH ranges from 1.9-6.0, then increases with increasing pH.[132] Citric acid buffers inhibit color formation, but for phosphatase determinations an acetic acid-acetate buffer is sometimes necessary.[133]

Sodium sulfite is the most stable buffer. Color develops fully within 5 minutes at pH 4.0-4.7. If the pH ranges from 3.0-4.0, the color develops slowly within 30 minutes. Below pH 3.0, development of color takes even longer.

The complex is extractable with ether,[134] isobutyl alcohol,[135] butyl alcohol,[136] isoamyl alcohol,[137] and acetoacetic ester [138] before reduction in nonaqueous solution to molybdenum blue. Spectrophotometrically,[139] under these conditions there are two characteristic points of maximum absorption, one at 625-30 mμ and the other at 730 mμ.[140] The latter has the greater sensitivity and is commonly used. The ratio of mixed solvents in the final sample should be kept constant to avoid a change in the maximum.[141] The molybdenum-blue color develops to its maximum within 40 minutes in nonaqueous solution and is quite stable in the isobutyl alcohol-ethanol mixture for 19 hours.

Perchloric acid, whose phosphate impurities are negligible, is very suitable for oxidation of samples containing organic matter.[142] This eliminates undesirable fumes, prevents fading of the blue complex due to excess nitric acid, and lessens the loss of phosphoric acid during evaporation. To minimize the inhibiting effect of excess acid on the blue complex and obtain more accurate results, the acid is neutralized to methyl red or phenolphthalein with ammonium hydroxide, and then the

[132] R. E. Kitson and M. G. Mellon, *ibid.* **16**, 466-9 (1944).

[133] Erling Lundsteen, *Enzymologia* **5**, 383-4 (1939).

[134] H. Copaux, *Compt. rend.* **173**, 656-8 (1921); L. D. Ras'kin, *Zavodskaya Lab.* **5**, 267-71 (1936); L. D. Ras'kin, D. T. Miroshnichenko and M. M. Bondarenko, *ibid.* **7**, 860 3 (1938).

[135] Isaac Berenblum and Ernst Chain, *Biochem. J.* **32**, 287-94; 295-8 (1938); W. A. Pons, Jr. and John D. Guthrie, *Ind. Eng. Chem., Anal. Ed.* **18**, 184-6 (1946).

[136] C. P. Sideris, *Ind. Eng. Chem., Anal. Ed.* **14**, 762-4 (1942).

[137] C. Rainbow, *Nature* **157**, 268 9 (1946).

[138] Karl Stoll, *Z. anal. Chem.* **112**, 81-90 (1938).

[139] Hans Borei, *Biochem. Z.* **314**, 351-8 (1943).

[140] W. A. Pons, Jr. and John D. Guthrie, *Ind. Eng. Chem., Anal. Ed.* **18**, 184-6 (1946).

[141] T. D Fontaine, *Ind. Eng. Chem., Anal. Ed.* **14**, 77 (1942); Harold W. Gerritz, *J. Assoc. Official Agr. Chem.* **23**, 321-54 (1940).

[142] Rex J. Robinson, *Ind. Eng. Chem., Anal. Ed.* **13**, 465-6 (1941).

solution made just acid with hydrochloric acid. A 720-mμ filter eliminates interference due to the methyl red indicator.

Since there is little specificity, it follows that many reducing agents have been used. Of these only a relatively small number are of practical interest. Experiments with sugars and metals as reducing agents[143] indicate that ammonium molybdate-sulfuric acid solutions in the presence of phosphate ions may be reduced by 5 per cent solutions of such sugars, as glucose, galactose, fructose, maltose, lactose, and sucrose. Metallic molybdenum, zinc dust, hydriodic acid,[144] sodium hydrosulfite, sodium thiosulfate,[145] ferrous sulfate,[146] ferrous ions and sodium bisulfite,[147] ammonium oxalate,[148] stannous chloride, hydrazine sulfate, phenylhydrazine hydrochloride, benzidine, gallic acid, 1,2,4-aminonaphthol sulfonic acid, *p*-aminophenol, *p*-aminophenol hydrochloride (Amidol),[149] monomethyl-*p*-aminophenolsulfate, *p*-methylaminophenol (Elon), hydroquinone, 8-hydroxyquinoline, ascorbic acid,[150] *p*-phenylenediamine and sodium sulfite,[151] and nitroso-2-naphthol in a sulfite-bisulfite reagent[152] give more or less satisfactory results. Metals such as magnesium, iron, and aluminum react slowly. Practically, every reductant has to be considered as a separate problem, often requiring different conditions due to differences in reduction potential.[153]

Several of the more important reducing agents will be discussed in more detail, including interferences with each. The first to be considered is stannous chloride,[154] the most widely used agent. It is variously designated as stannous chloride, chlorostannous acid, or even tin and hydrochloric acid even though the tin has completely dissolved. The use of stannous chloride permits both high and low molybdate concen-

[143] Chien-Pen Lo and Lucy Ju-Yung, *ibid.* **16**, 637 (1944).

[144] W. I. M. Holman, *Biochem. J.* **37**, 256-9 (1943).

[145] M. Pesez, *J. pharm. chim.* **2**, 127 a (1942).

[146] James B. Sumner, *Science* **100**, 413 14 (1944).

[147] V. A. Romashchenko, *Zavodskaya Lab.* **11**, No. 1, 104 (1945).

[148] L. D. Ras'kin, D. T. Miroshnichenko and M. M. Bondarenko, *ibid.* **7**, 860-3 (1938).

[149] Russell J. L. Allen, *Biochem. J.* **34**, 858-65 (1940).

[150] R. Ammon and K. Hinsberg, *Z. physiol. Chem.* **239**, 207-16 (1936).

[151] Iginio Napoli, *Ann. chim. applicata* **27**, 258-62 (1937).

[152] F. I. Nadler, *Biokhimiya* **9**, 376 8 (1944).

[153] G. A. Butenko and N. V. Kirsh, *Zavodskaya Lab.* **9**, 555-8 (1940).

[154] Osmond, *Bull. soc. chim. biol.* **47**, 745 (1887); C. W. Eddy and Floyd DeEds, *Ind. Eng. Chem., Anal. Ed.* **9**, 12-14 (1937); Isaac Berenblum and Ernst Chain, *Biochem. J.* **32**, 286 94, 295-8 (1938); S. R. Dickman and R. H. Bray, *Ind. Eng. Chem., Anal. Ed.* **12**, 665 8 (1940); J. T. Woods and M. G. Mellon, *ibid.* **13**, 760 4 (1941).

trations.[155] When the molybdate is very low, the blue complex is formed in the presence of chlorate ions, as for example 0.1 per cent of potassium chlorate.[156] Added acetone aids in stabilizing the color. The greatest accuracy has been reported at 1 ppm.[157]

In the determination of 0.01-0.11 per cent of phosphorus in steel, the maximum deviation using stannous chloride as the reducing agent is 0.003 per cent. Photometric determination of 0.001 mg. in a final volume of 50 ml. is feasible. A final concentration of about 1:16 hydrochloric acid, 0.3 per cent of ammonium molybdate, and 0.012 per cent of stannous chloride gives results which conform to Beer's law up to 0.5 ppm. of phosphorus. If sulfuric acid is used, the concentration should be maintained at 1:16 to 1:20 to obtain results that vary less than 2 per cent.[158] With the same reagent a ratio above 1:32 is necessary to prevent the appearance of color with no phosphorus in solution. Photometrically the peak in the absorption band is at 820 mμ.

Stannous chloride, even in strong hydrochloric acid, sometimes loses its reducing power unless tin is present. The result is that the samples may show a greenish tinge. The reagent may be kept under hydrogen to retard oxidation,[159] but the preparation of fresh solutions from stannous chloride is preferable.

Large amounts of carbon, manganese, sulfur, silicon, molybdenum, chromium, nickel, vanadium or tungsten do not interfere. Anions other than that of the acid used in the determination tend to interfere. The presence of more than 1 ppm. of ferric ion as sulfate decreases the color intensity and causes a green tinge. Dilution of the solution to contain 0.1 ppm. of phosphorus,[160] or addition of larger amounts of reducing agent,[161] are remedies. With over 30 ppm. of ferric ion, substantial amounts of additional stannous chloride are required. Either remedy may lead to a loss in accuracy. Interference of ferric ion up to 200 ppm. is eliminated by heating the acid solution for 1 hour at 100° with 5 ml. of 16 per cent sodium metabisulfite. Interference by ferric ion is also avoidable by heating the sample solution, about 1:70 with sulfuric acid, on a boiling water bath in the presence of 0.01 gram of aluminum foil for

155 B. R. Bertramson, *Soil Sci.* 53, 135-41 (1942).

156 P. J. Hardwick, *Analyst* 68, 183-4 (1943).

157 C. E. Beauchamp, *Mem. 15th conf. anual, Asoc. tec. azucar. Cuba* 1941, 47-51.

158 T. D. Fontaine, *Ind. Eng. Chem., Anal. Ed.* 14, 77-8 (1942).

159 G. R. Smith, W. J. Dyer, C. L. Wrenshall and W. A. DeLong, *Can. J. Research* 17B, 178-91 (1939).

160 *Ibid.*

161 S. R. Dickman and R. H. Bray, *Ind. Eng. Chem., Anal. Ed.* 12, 665-8 (1940).

30 minutes.[162] Another method for dealing with this interference is to reduce to the blue color and thereafter extract the color with butyl alcohol.[163] Extraction of the yellow phosphomolyhdatc with an immiscible solvent and reduction of this by shaking with stannous chloride solution are preferable. Thus orthophosphate is determined in the presence of pyrophosphate ion by treating the slightly acid sample with ammonium molyhdate followed hy a 1:3 hutyl alcohol-chloroform mixture. This method extracts 0.004-2.0 per cent of phosphate ion without interference from silicates and arsenates.[164]

The next reducing agent in importance is 1,2,4-aminonaphthol sulfonic acid, one less sensitive to interfering ions,[165] especially ferric ion, than stannous chloride. As is usual, low acidity results in reduction of molybdate without phosphate, and high acidity retards the development of color. The effective range for which Beer's law applies is 0.2-10 ppm. of phosphorus. Minimum transmittance is at 820 mμ. Aminosulfonic acid reacts initially at a higher concentration of phosphorus than stannous chloride. The method using the sulfonic acid is therefore generally less sensitive. The 2,5,7; 1,4,8; and 2,6,8 isomers are less effective. The 1,2,4 isomer is least sensitive to variation in the amount of reductant.

Interference due to a high sodium chloride content in the sample is overcome by addition of excess perchloric acid.[166] The use of 2-4 ml. of 60 per cent perchloric acid per 25 ml. of sample is sufficient to prevent any excess ammonium molyhdate from heing reduced because of insufficient acid, yet not enough to inhihit reduction of phosphomolyhdate. A working temperature of 25° ± 4° is desirahle, although the solution may he heated.[167] The optimum conditions for reduction are pH 0.05-0.6 and a 20-minute reduction time.[168] The addition of the reagents to a solution that has already heen diluted close to its final volume eliminates turbidity. An accuracy of 0 001 per cent of phosphorus pentoxide is obtainahle.

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[162] Rubens Salomé Pereira, *Rev faculdade med. vet, Univ. São Paulo* (Brazil) **1**, 153 69 (1939).

[163] C. P. Sideris, *Ind. Eng Chem., Anal. Ed.* **14**, 762-4 (1942).

[164] R. V. Mervel, *Zavodskaya Lab.* **11**, 135 7 (1945).

[165] A. D. Marenzi, *Anales farm bioquím.* (Buenos Aires) **10**, 64-9, 70-5 (1939); Adela Manussevich, *Rev. quím. farm.* (Santiago, Chile) **2**, No. 20, 2-3 (1944).

[166] James J. Rae and Edna V. Eastcott, *J. Biol. Chem.* **129**, 255 62 (1939); Mildred S. Sherman, *Ind. Eng. Chem., Anal. Ed.* **14**, 182-5 (1942).

[167] B. L. Horecker, T. S. Ma, and Erwin Haas, *J. Biol. Chem.* **136**, 775-6 (1940).

[168] W. L. Holmes and I. Motzok, *Sci. Agr.* **27**, 245 50 (1947).

A not unrelated reductant is monomethyl-*p*-aminophenolsulfate,[169] which is stable and low in cost. Interference from silica, chloride, sulfate, carbonate, and hydroxide ions is negligible. As little as 10 ppm. of phosphate ion in the presence of as much as 1000 ppm. of silica can be determined with this reagent within a 1 per cent error.[170]

The classical reducing agent is hydroquinone,[171] now of declining importance. Nevertheless it is still used as the official AOAC reductant and in many other cases. When the method was originally developed, alkaline solutions were used, but acidification and heating give better results. By close control of the acid concentration, the determination may be carried out in the presence of silica with this reducing agent. The optimum pH in the absence of silica is 5.2, adjusted by a sodium sulfite or sodium succinate buffer. Above a pH of 5.8 the color of the solution begins to fade on standing.[172]

Variation in the concentration of the molybdate, hydroquinone, and sulfite reagents does not affect the color produced. The sulfite reagent must be added last to develop the color fully. If the determination is made within the isoelectric range of the phosphomolybdate and molybdate ions, where reactivity is at a minimum, the greatest color stability is obtained.[173] A minimum in transmittance is at 460 mμ.[174]

The transmittance is usually read at 650 mμ and Beer's law is valid. Barium, bismuth, gold, lead, mercury, silver, stannic tin, strontium, thorium, titanium, and zirconium precipitate under this procedure. Arsenites may be present to 50 ppm. and arsenates to 100 ppm.

The blue complex obtained on reduction with hydrazine sulfate[175]

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169 M. Popp and H. Westerhoff, *Bodenkunde u. Pflanzenernähr.* 4, 19-29 (1937); G. A. Butenko and N. V. Kirsh, *Zavodskaya Lab.* 9, 555-8 (1940); J. Schnell and R. Frisch, *Bodenkunde u. Pflanzenernähr.* 21/22, 341-3 (1940); G. Gomori, *J. Lab. Clin. Med.* 27, 955-60 (1942).

170 George A. Johns, *Power* 86, 112 (1942).

171 Richard D. Bell and Edward A. Doisy, *J. Biol. Chem.* 44, 55-67 (1920); G. Barac, *Bull. soc. chim. biol.* 20, 1278-81 (1938); 21, 139-42 (1939); Leon Giraut-Erler, *Compt. rend. soc. biol.* 134, 507-9 (1940); L. S. Stoloff, *Ind. Eng. Chem., Anal. Ed.* 14, 636-7 (1942); T. S. Harrison and H. Storr, *J. Soc. Chem. Ind.* 63, 154-7 (1944).

172 R. E. Kitson and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 16, 466-9 (1944).

173 L. S. Stoloff, *ibid.* 14, 636-7 (1942).

174 R. E. Kitson and M. G. Mellon, *ibid.* 16, 466-9 (1944).

175 Herman J. Morris and Herbert O. Calvery, *Ind. Eng. Chem., Anal. Ed.* 9, 447-8 (1937); M. S. Saravi, *Rev. col. farm. nac. Rosario* 5, 219 (1938); John L. Hague and Harry A. Bright, *J. Research Natl. Bur. Standards* 26, 405-13 (1941); W. J. Boyer, *Proc. Am. Soc. Testing Materials* 44, 774-6 (1944); Max Herzog, *Chemist-Analyst* 33, 4-7 (1944); W. B. Sobers, *Am. Foundryman* 6, No. 9, 2-4 (1944); D. F. Boltz and M. G. Mellon, *Anal. Chem.* 19, 873-7 (1947).

is stable for 24 hours. The pH must be kept below 1 to prevent reduction of molybdic acid.[176] Colored ions interfere unless proper compensation is made. Beer's law holds up to 0.11 per cent of phosphorus within an experimental error of 0.003 per cent of phosphorus. Maximum absorption occurs at 830 mμ, but Beer's law is effective both at 830 and 650 mμ.

Reduced molybdate as a reductant is of minor importance.[177] Ascorbic acid as the reducing agent gives the maximum blue color in 7 minutes at 37°.[178] The corresponding arsenic complex remains colorless. With a 1 per cent solution of diaminophenol hydrochloride, or Amidol, in 20 per cent sodium bisulfite solution as reductant, the color is constant for 5-30 minutes over a temperature range of 8-26°.[179]

In general in the varied methods of reduction to molybdenum blue, arsenites do not interfere as much as arsenates.[180] Reduction for 1 hour at 50° causes variations in the instrument readings.[181] Under 0.03 per cent of arsenic does not interfere;[182] over that it is best to remove it by boiling a perchloric acid solution of sample with 5 ml. of 1:4 hydrobromic acid, volatilizing the arsenic as the bromide. Fluoride ions should be removed to eliminate interference,[183] either by evaporation with perchloric acid[184] or by adding an approximate equivalent of boric acid to the aliquot of sample to form the fluoborate ion.[185] For 0.3 mol of fluoride ion per liter, add 15 ml. of 5 per cent boric acid. Interference from silica is overcome by the use of a molybdate reagent with a high sulfuric acid content.[186]

When sodium sulfite is added to the sample, a red-brown color often results. By heating to boiling for about a half minute this will disappear. This is necessary only where a blank is being read on the solution before addition of the reagent as it will disappear later when the solution is heated with the reagent. A stock solution for permanent standards

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[176] W. R. Shelton and Horace J. Harper, *Iowa State Coll. J. Sci.* 15, 403-13 (1941).

[177] F. Giesecke, G. Michael and L. Schulte, *Bodenkunde u. Pflanzenernähr.* 7, 171-3 (1938); H. W. Gerritz, *J. Assoc. Official Agr. Chem.* 22, 131-7 (1939).

[178] R. Ammon and K. Hinsberg, *Z. physiol. Chem.* 239, 207-16 (1936).

[179] Russell J. L. Allen, *Biochem. J.* 34, 858-65 (1940).

[180] Lionel B. Pett, *Biochem. J.* 27, 1672-6 (1933).

[181] Russell J. L. Allen, *ibid.* 34, 858-65 (1940).

[182] A. J. Bursuk, *Zavodskaya Lab.* 8, 12-16 (1939).

[183] J. T. Woods and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 13, 760-4 (1941).

[184] S. R. Dickman and R. H. Bray, *Soil Sci.* 52, 263 (1941); Rex J. Robinson, *Ind. Eng. Chem., Anal. Ed.* 13, 465-6 (1941).

[185] L. T. Kurtz, *Ind. Eng. Chem., Anal. Ed.* 14, 855 (1942).

[186] Charles E. Brambel and R. P. Cowles, *Science* 85, 340-2 (1937).

ammonium molybdate solution. Dilute to volume, mix, and after 15 minutes read with a red filter or at 750 mμ.[192]

Hydroquinone.[193] Prepare the following reagents. Dissolve 25 grams of ammonium molybdate in 300 ml. of water. Dilute 75 ml. of concentrated sulfuric acid to 200 ml. and add to the molybdate solution. Dissolve 0.5 gram of hydroquinone in 100 ml. of water and add a drop of concentrated sulfuric acid to retard oxidation.

Dilute an aliquot of sample containing about 0.02-0.08 mg. of phosphorus to about 5 ml. in a 10-ml. volumetric flask. At the same time take a standard containing 0.05 mg. of phosphorus in another such flask. To each add 1 ml. of the ammonium molybdate reagent. Agitate to mix and allow the flasks to stand for a few moments. Add 1 ml. of the hydroquinone reagent and again agitate the flasks. Add 1 ml. of 20 per cent sodium sulfite solution. Dilute to volume with water, stopper the mouth of the flask with the thumb or forefinger, and shake to mix the contents thoroughly. Allow to stand for 30 minutes and compare with a standard treated simultaneously and in identical manner. If the concentration of phosphorus in the unknown differs from the standard by more than 40 per cent, repeat using a larger or smaller aliquot. The color may also be read photometrically.

Alternatively,[194] transfer to a 25-ml. volumetric flask an aliquot containing up to 0.3 mg. of phosphorus and adjust the volume to 10-15 ml. Add 2 ml. of 5 per cent ammonium molybdate in 1:35 sulfuric acid. Mix well and add 2 ml. of 0.5 per cent hydroquinone solution made acid with 1 drop of concentrated sulfuric acid per 100 ml. Mix well and add 2.5 ml. of 20 per cent sodium succinate solution. Dilute to volume, mix, and read at 460 mμ within 4 hours. After a time, succinate may crystallize out at this pH. The reactions appear to be instantaneous. An alternative buffer is 3 ml. of 20 per cent sodium sulfite solution.

Hydroquinone in the presence of Silicates.[195] Neutralize an aliquot of clear sample containing 0.01-0.1 mg. of phosphorus, with 1:20 sulfuric acid. Add 7 ml. of 1:20 sulfuric acid. Add, with thorough agi-

[192] Adele Manussevich, *Rev. quím. farm.* (Santiago, Chile) 2, No. 20, 2-3 (1944); A. D. Marenzi, *Anales farm. bioquím.* (Buenos Aires) 10, 64-9, 70-5 (1939).

[193] Official and Tentative Methods of Analysis of Association of Official Agricultural Chemists. Fifth Edition. pp. 127-8. Association of Official Agricultural Chemists, Washington, D. C. (1946).

[194] L. S. Stoloff, *Ind. Eng. Chem., Anal. Ed.* 14, 636-7 (1942).

[195] T. S. Harrison and H. Storr, *ibid.* 63, 154-7 (1944).

tation after each addition, 2 ml. of a 5 per cent solution of ammonium molybdate in 1:20 sulfuric acid, 2 ml. of 5 per cent hydroquinone solution containing 1 ml. of 1:20 sulfuric acid per 100 ml., and 3 ml. of fresh 20 per cent sodium sulfite solution. Mix well and add 7 ml. of 4 per cent sodium hydroxide solution. Dilute to 50 ml. and determine the phosphorus content colorimetrically or by comparing with a calibration curve, determined at 610 mμ.

Hydrazine Sulfate.[196] Dilute or concentrate to 10 ml. an aliquot of sample containing 0.005-0.05 mg. of phosphorus. Add 15 ml. of 10 per cent filtered sodium sulfite solution. Heat gently to boiling and boil for 1-2 minutes. Cool rapidly to 20° and transfer to a 50-ml. volumetric flask.

Prepare an ammonium molybdate–hydrazine sulfate–sodium sulfite reagent as follows: Dissolve 20 grams of ammonium molybdate in 500 ml. of water and 300 ml. of sulfuric acid and dilute to 1 liter. To 25 ml. of this solution diluted to 60 ml., add 10 ml. of 0.15 per cent hydrazine sulfate solution and 20 ml. of 10 per cent sodium sulfite solution.

Add 20 ml. of this reagent to the contents of the flask and dilute to volume. Measure the transmittance due to other ions in a portion and return this to the flask. Warm for 8-10 minutes in a boiling water bath to develop the molybdenum-blue color, cool to 20° and adjust to volume. Mix and measure the decrease in transmittance at 830 mμ due to the molybdenum-blue color.

Alternatively, to an aliquot of sample containing 0.005-0.05 mg. of phosphorus add 15 ml. of 10 per cent sodium sulfite solution. Heat to boiling and boil gently for 20-30 seconds.

Prepare a fresh reagent by diluting 25 ml. of 4 per cent ammonium molybdate solution to 80 ml., adding 10 ml. of 0.15 per cent hydrazine sulfate solution and diluting volumetrically to 100 ml.

To the sample solution, add 20 ml. of the reagent, heat to 90°, and digest at that temperature for 4-5 minutes. Heat just to boiling, remove from the heat, and cool rapidly to room temperature. Transfer to a 50-ml. volumetric flask by draining but not washing and dilute volumetrically to 50 ml. with 1:4 diluted reagent. Determine colorimetrically by comparison with a similarly prepared blank and standard or against a calibration curve.

[196] W. J. Boyer, *Proc. Am. Soc. Testing Materials* 44, 774-6 (1944).

Orthophosphate as the Phosphovanadomolybdate Complex

When an excess of molyhdate solution is added to an acidified solution of a vanadate and an orthophosphate, a yellow coloration results because of the formation of what is probably molyhdivanadophosphoric acid.[197] The formula $(NH_4)_3PO_4 \cdot NH_4VO_3 \cdot 16MoO_3$ has heen tentatively suggested for this complex, although the exact composition is uncertain. For very precise work the samples should he run at a constant room temperature.[198] Otherwise the determinations should be made within the range 20-30°. The use of a mercury vapor lamp provides a monochromatic light source which simplifies the photoelectric determination even further, presenting a linear relationship that is steeper in slope.[199]

The color of the phosphovanadomolybdate complex is more stable than that of the molyhdenum hlue complex. Determination hy means of this method generally applies to a wider range of concentration and is especially desirahle if ferric and silicate ions are present, hoth of which often interfere in the molyhdenum hlue method. If comparison is made hy visual means, the molybdenum blue color is easier to read. A moderate excess of ammonium vanadate and of ammonium molybdate is sufficient for color development; heyond that more reagent has no effect on the intensity other than to introduce a slight color due to the vanadate solution. It is advisahle to make up the ammonium vanadate reagent in perchloric acid rather than nitric acid, to avoid interference in the transmittance reading.[200]

Perchloric acid is the desirable acidifying agent in concentrations equivalent to 13-17 ml. of 72 per cent perchloric acid in 100 ml. of solution. Too much acid prevents full color development and too little does not prevent the formation of a precipitate on the addition of ammonium molyhdate. Working near the upper limit of the range hastens the dehydration of silica without significantly altering results from phosphate.

Nitric acid is also suitable but acid must he added in excess of 1:75

[197] G. Misson, *Chem.-Ztg.* 32, 633 (1908); *Ann. chim. anal. chim. appl.* 4, 267-9 (1922); W. M. Murray, Jr. and S. E. Q. Ashley, *Ind. Eng. Chem., Anal. Ed.* 10, 1-5 (1938); G. Bogatzki, *Arch. Eisenhuttenwes.* 12, 539-42 (1938-9); cf. Warren C. Vosburgh and Gerald R. Cooper, *J. Am. Chem. Soc.* 63, 437-42 (1941).

[198] E. John Center and Hobart H. Willard, *Ind. Eng. Chem., Anal. Ed.* 14, 287-8 (1942).

[199] E. J. Vaughan, *J. Proc. Roy. Inst. Chem. Gt. Brit. Ireland* 1943, 214.

[200] J. A. Brabson, J. H. Karchmer and M. S. Katz, *Ind. Eng. Chem., Anal. Ed.* 16, 553-4 (1944).

to prevent the appearance of the orange-yellow color which forms in neutral or slightly acid solution. The color of the phosphovanadomolybdate complex does not vary up to 1:9 acid, except that it develops more slowly. Above 1:9 the development of color is so slow that it may lead to error. The addition of ammonia then produces a deeper color due to a reduction in the concentration of free acid. Sulfuric and hydrochloric acids act similarly.

Interference by large amounts of ferric ion can be prevented by addition of sodium fluoride.[201] The tungsten and vanadium content should be low to prevent tungstic and vanadic acid precipitates from occluding phosphorus pentoxide. Silicates in large amounts interfere because of the formation of yellow silicomolybdic acid. Treatment of a high-silica content sample with perchloric acid dehydrates the silica and renders it easy to filter.[202]

The greater the amount of evaporation before the addition of acid, the steeper the slope obtained in plotting a calibration curve. By carefully evaporating the acid solution to dryness, without overheating, all excess acid may be removed, and the concentration controlled by adding a constant amount of dilute acid.[203] This yields a consistently steeper curve.

The time for development of color varies from 4 [204] or 5 [205] minutes to 30 minutes.[206] The latter time interval is usually employed and is particularly necessary if bismuth, thorium, arsenate, chloride, and fluoride ions are present, since they delay the development of color. Heating in some cases hastens the appearance of full color, but this may be done only if silicates and arsenates are absent.

Alloy steels and cast irons with a high nickel, chromium, or copper content and low phosphorus content are determined by difference, obtained by subtracting the reading on the sample without reagent from that obtained after adding reagent to the sample. This compensates for absorption due to other elements.[207] Converting iron to the perchloride removes the characteristic yellow color of ferric chloride, shifting the

[201] G. Bogatzki, *Arch. Eisenhuttenw.* 12, 195-8 (1938).

[202] Hobart H. Willard and E. John Center, *Ind. Eng. Chem., Anal. Ed.* 13, 81-3 (1941).

[203] T. S. Harrison and W. Fisher, *J. Soc. Chem. Ind.* 62, 219-21 (1943).

[204] Hobart H. Willard and E. John Center, *Ind. Eng. Chem., Anal. Ed.* 13, 81-3 (1941).

[205] R. E. Kitson and M. G. Mellon, *ibid.* 16, 379-83 (1944).

[206] Ruth Adele Koenig and C. R. Johnson, *ibid.* 14, 155-6 (1942); W. M. Murray, Jr. and S. E. Q. Ashley, *ibid.* 10, 1-5 (1938).

[207] T. S. Harrison, *J. Soc. Chem. Ind.* 63, 350-1 (1944).

region of maximum transmittance to the ultraviolet, and permits the measurement of the yellow phosphovanadomolybdate complex without interference. The selection of wave length at which phosphorus is determined is also important. At 550 mμ a variation in phosphorus concentration makes little difference in the transmittance, whereas, near the ultraviolet, interference from iron increases. Readings of about equal accuracy are obtainable at 430 mμ, 450 mμ, and 470 mμ.[208]

Copper nitrate shows a slight absorption at 420 mμ so that a similar amount should be provided in the standards. Iron causes a slight interference which is avoided by use of an aliquot of sample as a blank to which all reagents except molybdate have been added.[209] Silver may interfere by causing turbidity. Ceric and stannic ions precipitate. Ferrous, sulfide, thiosulfate, and thiocyanate ions reduce the complex to molybdenum blue. The color usually is fully developed within 5 minutes and is stable for 1 hour. Beer's law applies up to 40 ppm. for measurements made at 460 mμ.[210] By suitable dilution of the sample, photometrically satisfactory results are obtained for the range 0.1-1.0 per cent of phosphorus.[211] The accuracy can be better than 0.01 per cent for samples containing 0.14-0.18 per cent of phosphorus pentoxide.[212]

Procedure.[213] *Silica Absent.* Transfer a sample containing 0.05-0.5 mg. of phosphorus. Heat nearly to boiling, add 10 ml. of 0.25 per cent ammonium vanadate solution, and let cool. Add 20 ml. of 5 per cent ammonium molybdate solution and mix well. Transfer to a 100-ml. volumetric flask, filtering unless absolutely clear. Dilute to volume, mix, and read the transmittance around 470 mμ.

Silica Present. Prepare a reagent by dissolving 2.345 grams of anhydrous ammonium metavanadate in 400 ml. of hot water. Add 14 ml. of 72 per cent perchloric acid, cool, and dilute to a liter. Measure out an aliquot of sample containing 0.08-2.0 mg. of phosphorus. If comparison is to be made visually with a series of standards, at least 0.005 mg. of phosphorus should be present in the aliquot. Adjust the acidity to be equivalent to a 17-ml. excess of 72 per cent perchloric acid. Warm

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208 Ruth Adele Koenig and C. R. Johnson, *Ind. Eng. Chem., Anal. Ed.* 14, 155-6 (1942).

209 "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 334-7. American Society for Testing Materials, Philadelphia, Pa. (1946).

210 R. E. Kitson and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 16, 379-83 (1944).

211 W. M. Murray, Jr. and S. E. Q. Ashley, *ibid.* 10, 1-5 (1938).

212 J. A. Brabson, J. H. Karchmer and M. S. Katz, *ibid.* 16, 553-4 (1944).

213 R. E. Kitson and M. G. Mellon, *ibid.* 16, 379-83 (1944).

the solution to about 90° and add 10 ml. of the ammonium vanadate solution. Wash the sides of the beaker and watch glass with about 15 ml. of water. Mix, cool to room temperature, and filter into a 100-ml. volumetric flask. This removes silica. Wash the beaker and paper with three 10-ml. portions of water, keeping the volume in the flask under 90 ml. Cool to 25°, shaking continuously. Add 7.5 ml. of freshly filtered 10 per cent ammonium molybdate solution and dilute to volume. Mix thoroughly and allow to stand for 30 minutes. Determine the transmittance or compare with standards.

Orthophosphate as the Phosphomolybdate Complex

The reaction in the preceding method gives only the molybdate if the ammonium metavanadate is omitted. This is extractable into butanol or mixtures of solvents with butanol.

Procedure. Transfer 5 ml. of sample containing 0.005-0.05 mg. of phosphorus, and an equivalent standard, to separatory funnels. Approximately neutralize to litmus. To each add 2.5 ml. of 1:17 sulfuric acid and 2.5 ml. of 5 per cent ammonium molybdate solution. Add 10 ml. of isobutanol and shake thoroughly. Other extractants may be substituted. Discard the aqueous layer and wash the isobutanol layer twice with 5-ml. portions of 1:35 sulfuric acid. Compare.

Orthophosphate by Strychnine Molybdate

Orthophosphate ions treated with an acid strychnine molybdate reagent [214] produce a colloidal dispersion of strychnine phosphomolybdate.[215] Increasing the amount of precipitating reagent promotes an increase in turbidity and a decrease in transmittance readings up to the equivalent point.[216] The final turbidity is a function of the concentration of strychnine, orthophosphate ion, and molybdate ion. Determinations are also made by adding equal amounts of reagent and of 10 per cent gum arabic solution. From 0.0002-0.001 mg. per ml. of phosphoric acid may be determined nephelometrically by comparison with a series of standards or with a calibration curve. If the sample is

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[214] I. Pouget and D. Chouchak, *Bull. soc. chim.* [3], 5, 104 (1909); *ibid.* [3] 9, 649 (1911).

[215] Eduard Rauterburg, *Mikrochemie* [2] 4, 467-82 (1932); Walter Koch, *Techn. Mitt. Krupp, Forschungsber.* 2, 37-46 (1938); *Arch. Eisenhüttenw.* 12, 69-80 (1939); F. Postic, J. Rabaté and J. Courtois, *J. pharm. chim.* 2, 122-5 (1942).

[216] M. L. Chepelevetskii, *Zavodskaya Lab.* 11, 498-503 (1945).

in dilute acid solution, even twice as great a proportion of arsenate, silicate, or acidic ions does not interfere. Corrections may be made for solutions containing colored inorganic ions.

In another modification, the precipitate formed with strychnine molybdate is dissolved in sodium carbonate and the red color which develops on heating with phenylhydrazine is measured. The presence of up to 8 mg. of citric acid does not interfere. The precipitate may be separated, redissolved, and determined by reaction with ferrocyanide.

Procedure. *Direct Comparison.* Prepare a strychnine molybdate reagent by mixing equal parts of a 1 per cent strychnine sulfate solution and a 1.5 per cent solution of ammonium molybdate in 1:2 nitric acid. Allow to stand for 24 hours and filter. This reagent is stable for at least 1 month.

To a 10-ml. aliquot of sample containing 0.01-0.1 mg. of phosphorus and a similar standard which contains the same acid and basic ions as the sample, add 1 ml. of the reagent, stirring vigorously during the addition. After 5 minutes, compare the resulting turbidity nephelometrically or, alternatively, compare the sample with a series of standards.

To prepare a stable series of artificial standards, add dropwise with vigorous stirring 2 ml. of tincture of benzoin to 200 ml. of water. Pour through several filter papers, retaining only the first turbid portion of the filtrate. Dilute to correspond to the desired concentrations of phosphorus as shown by natural standards. Transfer to tubes and seal.

Development with Ferrocyanide. To a sample containing 0.01-0.1 mg. of phosphorus add 2 ml. of the reagent drop by drop with shaking. Let stand with occasional shaking for 10 minutes and centrifuge at 1500 rpm. for 3 minutes. Decant, dry the mouth of the tube, and add 3 ml. of water. Wash the precipitate with this, centrifuge, and decant. Repeat the washing operation.

Dissolve the precipitate in 2 ml. of 1 per cent sodium hydroxide solution and wash into a 100-ml. flask with 28 ml. of water. Add 20 ml. of 20 per cent potassium ferrocyanide solution and 10 ml. of concentrated hydrochloric acid. Mix and let stand for 10 minutes. Dilute to volume and mix. Compare with a standard similarly prepared containing 0.05 mg. of phosphorus. Approximate adjustment of the relative volumes of sample and standard can be made by comparison of the volumes of centrifuged precipitate.

Meta- and Pyrophosphates by Measurement of Ferric Thiocyanate Color

The decrease in ferric thiocyanate color in the presence of a fixed amount of iron is directly proportional to the amount of metaphosphate and pyrophosphate in solution.[217] This reaction forms a basis for a method for the photometric and colorimetric determination of these ions in water.[218] Some other reagents also form complexes with ferric ions that are sensitive to the metaphosphate concentration. An example is ferron.[219]

Small amounts of metaphosphate and pyrophosphate are added to water to assist in the control of scale formation and corrosion. From 0.01-5 ppm. is the usual concentration for this purpose. The method outlined was developed chiefly to check concentrations of these salts. With higher pH values the transmittance increases rapidly, necessitating very close pH control. The decrease in ferric thiocyanate color due to metaphosphate ions is less influenced by pH than that due to pyrophosphate ions.

Ions that form complexes with thiocyanate decrease the fading of the ferric thiocyanate complex and thus interfere. This is discussed in much more detail under determination of iron by this method. Monovalent positive ions have no effect on fading of the ferric color by metaphosphate, divalent positive ions have a definite effect, and aluminum has a very marked effect. To illustrate, in a solution containing 1 ppm. of sodium metaphosphate, 136 ppm. of calcium ions introduce an error of 5 per cent, 600 ppm. of magnesium ions cause an error of 12 per cent, and 0.25 ppm. of aluminum results in an error of 15 per cent.

If the concentration of metaphosphate is between 1 and 30 ppm. and pyrophosphate ions are present, bring the pH to 0.5 with 5 ml. of 1:1.5 nitric acid per 100 ml. If both metaphosphate and pyrophosphate are present, determination is made at pH 0.5 and pH 2.0, the latter representing an acidity at which metaphosphate and pyrophosphate in the range 1-15 ppm. have about the same effect on ferric thiocyanate. For metaphosphate concentrations between 0.05 and 2 ppm. and for pyrophosphate concentrations between 0.05 and 1 ppm., extract the nitric acid-ammonium thiocyanate solution of sample with isoamyl alcohol-ethyl ether solution.

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[217] J. T. Woods and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 13, 551-4 (1941).
[218] Henry E. Wirth, *ibid.* 14, 722-5 (1942).
[219] Joseph J. Fahey, *ibid.* 11, 362-3 (1939).

Standards are prepared from metaphosphate- and pyrophosphate-free water of the same composition as that tested. Photometrically the color is determined with a 30-mμ slit centered at 470 mμ. Results are accurate to 0.5 per cent.

Procedure. Dilute the sample if necessary so that it will contain 0.05-2 ppm. of sodium metaphosphate or 0.05-1 ppm. of sodium pyrophosphate. To 90 ml. of sample add standard ferrous ammonium sulfate solution (page 307) so that the total iron concentration is 0.02 mg. Using a glass electrode, adjust the pH of the sample to 3.0 with 1:40 nitric acid. Add 5 ml. of 20 per cent ammonium thiocyanate solution which has been freed of iron by three extractions with 5:2 isoamyl alcohol-ethyl ether solution. Dilute the test sample to 100 ml. and, after 5 minutes, compare with similarly treated standards.

To increase the sensitivity, shake the aqueous solutions that have been treated with ammonium thiocyanate with 15 ml. of 5:2 isoamyl alcohol-ethyl ether solution. Pipet out 5 ml. of the solvent layer and add 0.5 ml. of acetone to clear any turbidity. Compare this solution prepared from the sample with similar standards.

Miscellaneous

The yellow color of ammonium phosphomolybdate is colorimetrically determinable as a finely dispersed precipitate. As procedure, measure out 10-100 ml. of sample solution. Silica and titanium must be absent. Prepare a standard or a series of standards at the same time. Dilute each to about 90 ml. and add 5 ml. of 1:5 nitric acid and 4 ml. of a 5 per cent solution of ammonium molybdate. Compare after 3 minutes.

Another variation is to dissolve the phosphorus, separated as phosphomolybdate, in sodium hydroxide solution and convert the accompanying molybdenum to the sulfide. To a filtered and well-washed phosphomolybdate precipitate add 0.4 per cent sodium hydroxide solution from a calibrated pipet or buret until the precipitate is dissolved Add a measured excess which should be about half the amount required to dissolve the precipitate. Excess is essential or a black color is obtained with hydrogen sulfide. Dilute to a known volume and mix.

Take a suitable aliquot of the sample solution and dilute to about 25 ml. in a Nessler tube. Pass hydrogen sulfide through for 5 minutes. Saturation is essential for development of the proper color. Place in a boiling water bath for 5 minutes. Remove, cool, dilute to 50 ml., and compare with a standard. The color after heating is stable for 2 hours.

By preparation under properly standardized conditions, a colloidal dispersion of silver orthophosphate can be obtained suitable for nephelometric comparison. Make the sample solution slightly alkaline to phenolphthalein by careful addition of 20 per cent sodium hydroxide solution, noting the amount used. Make just acid with 1:100 sulfuric acid and cool. Neutralize with approximately 0.4 per cent sodium hydroxide solution. To the neutralized solution add 1 ml. of 10 per cent ammonium sulfate solution and 1.5 ml. of 0.4 per cent sodium hydroxide solution. Dilute to such a volume that the phosphorus content is about 0.005 mg. per ml.

Place 10 ml. of neutralized 2 per cent silver nitrate solution in each of two 25-ml. volumetric flasks. Add 10 ml. of standard and sample through funnels which have been drawn down to deliver 10 ml. in about 15 seconds. Gently rotate the flasks while the solution is being added. Rinse out the original containers with distilled water and pour the wash-water through the funnels. Rinse the funnels with distilled water, make up to volume, and read in the nephelometer. Chlorides must be rigidly excluded from all reagents used.

Sensitivity in determination of phosphate in aqueous solution can be obtained by precipitation with 8-hydroxyquinoline.[220] To the sample at 60° add a mixed reagent containing 8-hydroxyquinoline and ammonium molybdate. Heat at that temperature for 0.5 hour and let cool. Centrifuge and wash the precipitate with water. The compound contains 1 mol of phosphomolybdic acid:3 mols of 8-hydroxyquinoline. Dissolve the precipitate in 0.5 per cent sodium hydroxide solution and use as a sample for determination of phenol.[221] The color developed is about 5-10 times as great as for any phosphate method in use and agrees with results by the molybdenum blue method.

The yellow color produced by the reaction between orthophosphate and an ammonium molybdate-quinine reagent is used for colorimetric estimation. A suitable sample contains 0.002-0.02 mg. of phosphorus pentoxide. Iron must be absent. As reagent, dissolve 1 gram of quinine sulfate in 50 ml. of 1:5 nitric acid. Add a saturated solution of barium hydroxide until no further precipitation occurs. Filter and mix the filtrate with 40 grams of ammonium molybdate dissolved in 500 ml. of 1:1 nitric acid. Dilute to 1 liter and mix well. To the sample and a series of standards at about 45 ml. each, in Nessler tubes, add 2 ml. of 1:3 nitric acid and 2 ml. of reagent. Dilute each to 50 ml. and compare.

[220] Earl J. King and George E. Delory, *Biochem. J.* 31, 2046-8 (1937).
[221] Second Edition, Vol. II, p. 197, Third Edition, Vol. III in preparation.

An indirect method determines orthophosphate by uranium acetate and potassium ferrocyanide. The orthophosphate is first precipitated with uranium acetate and filtered. This is redissolved and the uranium estimated with potassium ferrocyanide. The amount of uranium precipitated may also be estimated from that remaining in solution and the phosphorus calculated.

To 2 ml. of sample add 2 ml. of uranium acetate solution containing 2.1215 grams per liter. Prepare a solution of 105 grams of ammonium acetate and 100 ml. of glacial acetic acid per liter. Add 1 ml. of this mixture and dilute to 10 ml. with water. Centrifuge to settle the precipitate. For direct estimation decant and wash the precipitate twice wih 5 ml. of water to which 0.5 ml. of the acetate buffer has heen added. Dissolve the precipitate in 5 ml. of 5 per cent trichloroacetic acid, add to 2 ml. of 0.5 per cent potassium ferrocyanide solution, and compare with a standard amount of phosphate similarly treated. For estimation of excess uranium, pipet out 5 ml. of the clear supernatant liquid. Add this to 2 ml. of 0.5 per cent potassium ferrocyanide solution. Prepare a blank which has heen similarly treated, hut in which the sample has been suhstituted hy distilled water. Compare the two after 30 minutes. Calculate the amount of uranium acetate precipitated and multiply by 0.073 to give the result in terms of phosphorus, 0.167 in terms of phosphorus pentoxide, or 0.224 in terms of phosphate radical.

Orthophosphate ions, treated with hismuth oxyperchlorate, $BiOClO_4$, in the presence of an excess of perchloric acid to prevent hydrolysis, form a bismnth orthophosphate precipitate.[222] The phosphorus content may he determined from the turhidity.

The decrease in color of ferric salicylate is a method of estimation of phosphate.[223] Suitable samples are soils and nonferrous metals. Oxalates, glycolates, acetates, lactates, phthalates, formates, sulfates, citrates, and arsenates should be ahsent. A suitable sample is 0.1-2 mg. of phosphorus as phosphate made yellow to dinitrophenol with 1:1 hydrochloric acid, treated with 25 ml. of 0.3 per cent sodium salicylate and 25 ml. of 0.3 per cent ferric chloride solution in 0.1 N hydrochloric acid, and diluted to 250 ml.

The amount of elementary phosphorus in oil solution diluted with an alcohol-ether-acetone mixtnre can be estimated from the brown color produced with silver nitrate dissolved in acetone. The color is completely developed in 15 minutes. A trace of water promotes defloccula-

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222 M. L. Chepelevetskiĭ, *Zavodskaya Lab.* **11**, 493-503 (1945).

223 G. Kortum and M. Kortum-Seiler, *Reichsamt Wirtscaftsausbau, Chem. Ber.* Pruf Nr. 093 (PB52020), 1056-62 (1942).

tion. The standards, properly sealed, are stable. Dilute 1 ml. of sample to 10 ml. with a mixture of 40 ml. of ether, 20 ml. of ethanol, and 5 ml. of acetone. Add 0.2 ml. of a solution of 0.25 gram of silver nitrate in 100 ml. of acetone. To 9 ml. of the solvent mixture add 0.2 ml. of the silver nitrate reagent and sufficient of a standard oil containing 0.1 mg. of phosphorus per ml. to give approximately the same color as that developed in the sample. This is used as an approximation. Dilute the sample with oil free from phosphorus, until it comes within the range of a series of standards containing 0.05-0.1 mg. of phosphorus per gram of oil. Repetition of the procedure on the properly diluted sample then gives the true value of the sample.

As little as 0.01 mg. of yellow phosphorus[234] can be determined. Steam distill the sample and catch the distillate in 8.5 per cent silver nitrate solution. Wash the precipitate of double salt, $Ag_3P \cdot 3AgNO_3$, and treat in a Gutzeit apparatus (page 188) with a mixture of zinc, copper sulfate solution, and 20 per cent sulfuric acid. The phosphine gas liberated produces a yellow stain on mercuric bromide paper analogous to the usual arsine stain.

[224] Sidney Kaye, *J. Lab. Clin. Med.* 28, 225-9 (1942).

CHAPTER 47

SILICA

Silica is not only present in nearly all minerals but in animal and plant organisms. It is present in the common metals and their alloys, either as such or as the elementary silicon. The latter is an important element in special alloys. Thus the wide distribution of silica or silicon leads to samples from many sources, both organic and inorganic. Silica is not toxic but in the lungs can cause silicosis, making its presence in dusts of importance.

The methods of determination closely parallel those for phosphorus. The oldest and best known is the development of a yellow silicomolybdate, just as with phosphorus, that can be reduced to molybdenum blue, which is more sensitive.

In dealing with silica determinations, the wide occurrence of the combined element is a disadvantage. It is readily dissolved to some variable extent from any glass apparatus used. As much of the procedure as possible is carried out in paraffin-lined apparatus or in platinum, hard rubber, plastic, and even quartz. Reagents generally contain small amounts of silica, and only a minor amount of contamination with airborne dust can cause considerable error.

Samples

Magnesium and Magnesium Alloys. Silica and silicon as a eutectic mixture with molten magnesium form silicides which are converted to silica by treatment with nitric acid. To a sample[1] of less than 2 grams containing 1-5 mg. of silicon, add 25 ml. of water and 5 ml. of saturated boric acid solution. Place the container in a cold water bath and slowly add 11.7 ml. of 1:4 sulfuric acid per gram of sample. Unless the solution is kept cold, silicon may be lost as hydride. Add 1 ml. excess of the 1:4 sulfuric acid. Add 0.1 gram of potassium persulfate to oxidize any reducing materials such as ferrous iron and let stand at least 10 minutes. Dilute to about 60 ml. and filter into a 100-ml. volumetric flask. Wash the residue on the paper until the volume in the flask is about

[1] "1946 Book of ASTM Methods of Chemical Analysis of Metals," pp. 327-28. American Society for Testing Materials, Philadelphia, Pa. (1946).

90 ml. and either use as sample for development of the yellow silicomolybdate if the silicon content is under 1 mg. or dilute to volume and use an aliquot.

Ash the paper with the residue at not over 500°. Add 0.1 gram of sodium carbonate and fuse at about 850°. Take up the cooled fusion in water by adding 1:4 sulfuric acid dropwise until it is no longer alkaline. Filter into a 100-ml. volumetric flask and wash the paper with water until the volume of filtrate is about 90 ml. Use this as a second sample and add the results obtained with the two solutions. If the sample contains less than 0.2 mg. of silica, the accuracy can be improved by addition of a standard amount to bring the determination into a range of greater sensitivity.[2]

As another method of obtaining the solution without loss of silicon,[3] dissolve 2 grams of sample by adding dropwise 50 ml. of 1:1 nitric acid. If copper is present in excess of 0.2 per cent, substitute 1:5 sulfuric acid. In the latter case, filter the copper residue and add 10 ml. of concentrated nitric acid to the filtrate. In either case, boil for 15 minutes to remove nitrogen oxides. Cool, transfer to a 100-ml. volumetric flask, and dilute to volume. Use 25-ml. aliquots for the determination of silicon by sodium sulfite reduction of the silicomolybdate complex. Dilute sulfuric acid which has been saturated with bromine may replace the nitric acid used to oxidize the sample.[4]

Aluminum and Aluminum Alloys. Aluminum is often dissolved in an acid mixture of 115 ml. of concentrated sulfuric acid, 200 ml. each of concentrated nitric and hydrochloric acids, diluted to 1 liter.[5] Chill-cast aluminum that contains as its chief impurities iron and silicon has silicon in solid solution in the aluminum unless the iron content is very high. When the sample is dissolved in mixed acids, some of the silicon in solid solution forms silicon hydride and may be lost. Hence aluminum which has been heated and quenched from a temperature above 500°, as well as that containing magnesium silicide, should be dissolved in sodium hydroxide solution.

As a typical technic,[6] dissolve 0.005-0.2 gram of sample in 15 ml. of

[2] K. M. Popov and M. L. Vegrin, *Zavodskaya Lab.* **6**, 502-3 (1937).
[3] A. J. Boyle and V. V. Hughey, *Ind. Eng. Chem., Anal. Ed.* **15**, 618-19 (1943).
[4] A. L. Davydov and O. A. Malinovskaya, *Zavodskaya Lab.* **9**, 964-7 (1940).
[5] H. V. Churchill, R. W. Bridges and M. F. Lee, *Ind. Eng. Chem., Anal. Ed.* **9**, 201-2 (1937); L. H. Callendar, *ibid.* **9**, 533-4 (1937). H. V. Churchill, R. W. Bridges and M. F. Lee, *ibid.* **9**, 534 (1937).
[6] J. J. Stumm, *Proc. Am. Soc. Testing Materials*, Preprint No. 37, 5 pp. (1944); cf. E. N. Vasenko, *J. Applied Chem.* (U.S.S.R.) **19**, 605-7 (1946).

20 per cent sodium hydroxide solution. When the reaction subsides, wash down the sides of the container and boil for 10 minutes. Pour the solution into 25 ml. of 1:5 nitric acid and 5 ml. of 1:1 sulfuric acid. Wash the original vessel with 5 ml. of 1:10 sulfuric acid and then with water. Heat to boiling to dissolve aluminum salts, cool to 20°, and adjust the pH to 1.4 by adding, dropwise, 0.5 per cent sodium hydroxide solution from a copper pipet. Transfer to a 200-ml. volumetric flask, dilute to volume with sulfuric acid which has been adjusted to pH 1.4, and mix for use of aliquots. Develop the yellow silicomolyhdate color and read photometrically.

Some alloys, if boiled for a prolonged period with sodium hydroxide, yield inconsistent extinction values and lower results than those obtained by gravimetric and spectrographic methods.[7] A variant in technic to avoid that is the addition of 30 per cent hydrogen peroxide to the sodium hydroxide solution used to dissolve the sample.[8] Any residual hydrogen peroxide is often removed with potassium permanganate followed by oxalic acid.

As applied to an alloy, if the sample[9] contains up to 1.5 per cent silicon, use a 0.5-gram sample; up to 15 per cent, use a 0.2-gram sample. To the aliquot in a nickel crucible add 4 grams of sodium hydroxide and 10 ml. of water, cover, and place in a cold-water bath to prevent vigorous action. When the reaction is no longer violent, boil gently for 10 minutes, cool, and transfer to 40 ml. of 1:3 sulfuric acid. Add 3 ml. of 10 per cent hydrogen peroxide, mix, and heat to the boiling point. Cool the clear solution, transfer to a 500-ml. volumetric flask, and dilute to volume. Use a 25-ml. aliquot for up to 1.5 per cent silicon and a 5-ml. aliquot up to 15 per cent silicon, either of which is diluted to 50 ml. for determination of silicon by reduction to molybdenum blue with aminonaphthol sulfonic acid. For silica in aluminum of over 99.99 per cent purity dissolve in 1:3 hydrochloric acid and 1:4 nitric acid. Dilute and use an aliquot for formation of the silicomolybdate.[10]

A modification[11] calls for filtration through a paper wet with 2 per cent sodium hydroxide solution before acidifying. The color developed[12]

[7] A. Staples, *Analyst* 67, 287 (1942).

[8] Hans Pinsl, *Z. Metallkunde* 27, 107-14 (1935); B. A. Scott, *Analyst* 67, 389 (1942).

[9] H. Cox, *Metallurgia* 33, 121-3 (1946).

[10] P. Urech, *Metal Ind.* (London) 70, 303-4 (1947).

[11] K. A. Vasil'ev and O. D. Barinova, *Zavodskaya Lab.* 4, 1163-70 (1935).

[12] R. Gadeau, *Ann. chim. anal. chim. appl.* 19, 64-8 (1937); P. Urech, *Helv. Chim. Acta* 22, 1023-36 (1939); W. H. Hadley, *Analyst* 66, 486-9 (1941); *ibid.* 67, 5-8 (1942); *ibid.* 70, 43-5 (1945); W. Stross, *Analyst* 69, 44-5 (1944).

may be either the yellow or blue. Results that are sometimes high when compared with those obtained from alumina solutions are possibly due to the fact that, since much more reagent is used for alumina solutions than for aluminum, the silica loss during dehydration of the alumina solution is compensated for by reagent impurity, whereas this does not hold for aluminum.[13]

Aluminum-copper-silicon Alloys. A solution was obtained in determination of copper from treatment of the alloy with sodium hydroxide (page 80). Copper was in the residue, aluminum and silicon in the solution. Dilute the solution to 100 ml. and take a 5-ml. aliquot. To this add 15 ml. of water and then about 10 ml. of 1:100 sulfuric acid, dropwise. This amount should be such that the aluminum hydroxide produced on partial neutralization has just been dissolved on complete mixing. Use the sample so prepared for development of the yellow silicomolybdate followed by its reduction with stannous chloride.

Steel.[14] For a sample containing not more than 5 per cent of silicon dissolve 0.5 gram in 70 ml. of hot 1:20 sulfuric acid. Cool and add 15 ml. of 2.5 per cent potassium permanganate solution. Boil for 5-10 minutes and add a 20 per cent ammonium bisulfite solution slowly with stirring until the violet color disappears and manganese dioxide is dissolved. Boil to drive off excess sulfur dioxide, cool, and dilute volumetrically to 250 ml. Use 20-ml. aliquots for the determination of silicon by reduction to molybdenum blue with stannous chloride. Hydrogen peroxide will satisfactorily replace the sodium bisulfite.[15] Although 1:20 sulfuric acid has been used to dissolve samples, the use of other acids, such as perchloric acid,[16] increases the range of the method for different types of steel.

For steel containing less than 0.6 per cent of titanium which will dissolve under the specified conditions, a simplified technic may be applied.[17] Treat a 0.1-gram sample with 15 ml. of 1:1 nitric acid and 1 ml. of 30 per cent hydrogen peroxide. Warm to not over 90°. Add saturated potassium permanganate solution dropwise until a permanent pink

[13] J. A. Brabson, I. W. Harvey, G. E. Maxwell and O. A. Schaeffer, *Ind. Eng. Chem., Anal. Ed.* 16, 705-7 (1944).

[14] Donald F. Clausen and Harold D. Roussopoulas, *Anachem News* 6, 41-4 (1946).

[15] A. I. Kokorin and K. D. Vasil'eva, *Zavodskaya Lab.* 12, 123-4 (1946).

[16] Jean Birckel, *Ann. chim. anal.* 25, 18-19 (1943).

[17] Hans Pinsl, *Z. Metallkunde* 27, 107 (1935); *Arch. Eisenhüttenw.* 9, 223-30 (1935).

color is obtained. Add 2 ml. of concentrated hydrochloric acid and heat until the solution is clear, except for insoluble matter from the original sample. Cool to 20°, transfer to a 100-ml. volumetric flask, and dilute to volume. Develop the yellow color as molybdate by a special method, allowing for iron (page 695). A correction factor must be applied if tungsten is present.

There are numerous modifications.[18] Thus to 0.2 gram of sample[19] in the form of chips or turnings, add 10 ml. of 1:3 hydrochloric acid and 10 ml. of 1:4 nitric acid. Warm the mixture slightly, if necessary, to dissolve. Avoid evaporation to prevent changing the pH of the solution. Cool and dilute volumetrically to 100 ml. If columbium or tungsten are present, filter through a dry paper before taking a 25-ml. aliquot for development of color. For stainless steel modify the preceding technic by treating a 0.2-gram sample in the form of chips or turnings with 10 ml. of 1:3 hydrochloric acid and 5 ml. of 1:4 nitric acid. As soon as the steel has dissolved, add 5 ml. more of 1:4 nitric acid and heat for 2-3 minutes. Cool, dilute volumetrically to 100 ml., and filter if necessary before taking a 25-ml. aliquot.

Tungsten Steels.[20] To a 0.25-gram sample in a platinum dish add 0.5 gram of ammonium fluoride and 12 ml. of 1:2 nitric acid. Warm gently to dissolve and add 1 gram of boric acid dissolved in 10 ml. of warm water. Warm gently for 5 minutes. Transfer to a 500-ml. calibrated flask. Add 12 ml. of 1:2 nitric acid and dilute to volume with water. Allow to settle and filter about 100 ml. for use of aliquots in the determination of silicon by reduction with ferrous ammonium sulfate.

Copper-base Alloys.[21] Use a 1-gram sample of not finer than 10-mesh sample, containing up to 0.20 per cent silicon. If the amount of silicon present is higher, use a smaller sample. Transfer to a platinum crucible and cover to prevent subsequent volatilization of silicon tetrafluoride. Treat with 10 drops of 48 per cent hydrofluoric acid and an amount of 1:2 nitric acid equivalent to 0.6 ml. for each 100 mg. of copper, and 6 ml. in excess. Hydrofluoric acid forms stable complexes

[18] Paul Klinger and Walter Koch, *Arch. Eisenhüttenw.* 11, 569-82 (1938); S. I. Malov, P. Ya. Yakovley and A. A. Eliseev, *Zavodskaya Lab.* 5, 665-7 (1936); A. L. Davydov, B. E. Reznick and Z. M. Vaisberg, *ibid.* 8, 1033-8 (1939); E. I. Grenberg, *ibid.* 9, 355 (1940).

[19] David Rozental and Hallock C. Campbell, *Ind. Eng. Chem., Anal. Ed.* 17, 222-4 (1945).

[20] C. H. R. Gentry and L. G. Sherrington, *J. Soc. Chem. Ind.* 65, 90-2 (1946).

[21] O. P. Case, *Ind. Eng. Chem., Anal. Ed.* 16, 309-11 (1944).

of these applications to minerals, samples are glass,[28] alumina cements,[29] and lime solutions.[30]

Synthetic or Natural Alumina.[31] Transfer a sample containing 0.1-0.4 gram of alumina to a platinum dish, cover with 4 grams of anhydrous sodium carbonate and 0.7 gram of boric oxide, and stir with a platinum wire to mix. Fuse at 1000° in a muffle furnace for about 15 minutes or until the melt is perfectly clear. Cool, cover the melt with 50 ml. of water, and digest on a steam bath until dissolved. Cool to room temperature and transfer the solution to a 250-ml. volumetric flask containing 50 ml. of water. Rinse the dish, add the washings to the flask, and add 10 ml. of 1:1 sulfuric acid. Dilute to volume and use 50-ml. aliquots.

Tungstic Acid.[32] Neutralize the solution with 1:5 hydrochloric acid to pH 4 and add about 0.4 ml. excess. This acidity is insufficient to precipitate tungstic acid, which would carry down silica with it. Develop the yellow color of silicomolybdic acid or molybdenum blue. Limits of half the amount of silica as phosphorus and not over 2.5 times as much arsenic are desirable.

Boiler Scale. A sample has been prepared for determination of aluminum (page 242). Use an aliquot for determination of silica by ammonium molybdate and aminonaphthol sulfonic acid.

Alternatively,[33] grind a representative sample to a very fine powder and dry at 105°. Transfer 40 mg. of dried material to a platinum crucible, place in an electric muffle, and raise the temperature slowly. Heat for 45 minutes or to constant weight at 950-1000° Fuse with 0.2 gram of potassium carbonate, covering the sample completely. Cool, place the crucible upright in a beaker, add 5 ml. of 1:40 hydrochloric acid, and heat on a hot plate for 4-6 minutes. Treat with an additional 10 ml. of

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[28] F. V. Tooley and C. W. Parmelee, *J. Am. Ceram. Soc.* 23, 304-14 (1940).

[29] V. T. Illiminskaya, *Zavodskaya Lab.* 8, 863 6 (1940).

[30] E. I. Nagerova and A. D. Petrova, *Vsesoyuz. Nauch.-Issledovatel. Inst. Tsement.* No. 17, 56 60 (1937).

[31] F. Ya. Galakhov, *Zavodskaya Lab.* 6, 1011-12 (1937); A. N. Miklashevskii. *bid.* 6, 1209-13 (1937); J. Raffin, *Ann. chim. anal.* 25, 56-7 (1943); J. A. Brabson. . G. E. Maxwell and O. A. Schaeffer, *Ind. Eng. Chem., Anal. Ed.* 16,

ernikhov and B. M. Dobkina, *Zavodskaya Lab.* 12, 922-6 (1946).

dsay and R. G. Bielenberg, *Ind. Eng. Chem., Anal. Ed.* 12, 460-3

carefully heating to boiling with 5 ml. of water. Transfer to a 50-ml. volumetric flask, add 2 drops of phenolphthalein solution, and titrate with 1:2.5 sulfuric acid. Take the reading and add that much acid in excess, plus 0.5 ml. more. Dilute to volume and use an aliquot for development of the color.

Alternatively,[24] fuse 0.1-0.5 gram of sample with 10 parts of sodium carbonate for 10 minutes in a platinum crucible. Neutralize with concentrated hydrochloric acid and dilute volumetrically so that the concentration of silica is 0.05-0.1 mg. per ml. and use 10-ml. aliquots.

Mine Dust.[25] Determination under these circumstances is important in relation to the silicosis hazard. Fuse a sample of suitable size according to the probable silica content with an excess of potassium bisulfate until no further reaction occurs. Treat the cooled melt with an excess of 20 per cent sodium hydroxide solution and add excess alkali until about 10 per cent of free sodium hydroxide is present. Boil vigorously until all the silica is in solution as sodium silicate and then filter. Dilute to a known volume and use an aliquot as sample. Add 1:1 hydrochloric acid to the sample until acid to methyl orange before use.

Limestone, Dolomite, or Cement.[26] Use a sample of 0.01-5 grams according to the expected silica content. Treat in a platinum crucible with excess of concentrated nitric acid, added dropwise down the side until the main reaction has ceased. The insoluble residue contains all the silicic acid. Dilute with water to about 4 times the volume and filter. Wash the residue on the filter with hot water and dry. Ignite the paper and cool. Add twice the volume of anhydrous sodium carbonate and fuse to a homogeneous melt. Cool and take up in water. Use this solution or an aliquot as a sample, neutralizing for use.

Clay.[27] Ignite the sample with sodium peroxide and pure carbon and then take up in water. Filter and wash well. Add 1:9 sulfuric acid to make the filtrate distinctly acid and use as sample for development of the yellow color with ammonium molybdate. In various modifications

[24] I. M. Korenman and L. A. Kozhukhin, *Zavodskaya Lab.* 9, No. 1, 43-5 (1940).

[25] S. R. Rabson, *S. African Mining Eng. J.* 55, 199-203 (1944).

[26] N. A. Tananaev and A. M. Shapovalenko, *J. Applied Chem.* (U.S.S.R.) 11, 352-4 (1938).

[27] K. A. Vasil'ev and B. A. Faktorovich, *Legkie metal.* 1937, *No.* 5-6, 31-2.

of these applications to minerals, samples are glass,[28] alumina cements,[29] and lime solutions.[30]

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[28] F. V. Tooley and C. W. Parmelee, *J. Am. Ceram. Soc.* 23, 304-14 (1940).

[29] V. T. Illiminskaya, *Zavodskaya Lab.* 8, 863-6 (1940).

[30] E. I. Nagerova and A. D. Petrova, *Vsesoyuz. Nauch.-Issledovatel. Inst. Tsement.* No. 17, 56-60 (1937).

[31] F. Ya. Galakhov, *Zavodskaya Lab.* 6, 1011-12 (1937); A. N. Miklashevskii, *ibid.* 6, 1209-13 (1937); J. Raffin, *Ann. chim. anal.* 25, 56-7 (1943); J. A. Brabson, I. W. Harvey, G. E. Maxwell and O. A. Schaeffer, *Ind. Eng. Chem., Anal. Ed.* 16, 705-7 (1944).

[32] Yu. A. Chernikhov and B. M. Dobkina, *Zavodskaya Lab.* 12, 922-6 (1946).

[33] F. K. Lindsay and R. G. Bielenberg, *Ind. Eng. Chem., Anal. Ed.* 12, 460-3 (1940).

1:40 hydrochloric acid and continue heating until solution is complete. Cool and wash the contents of the crucible into the beaker with small amounts of water. Neutralize to phenolphthalein paper with 0.5 per cent sodium hydroxide solution. A floc generally forms just before the neutral point is reached. Then add just enough 1:20 hydrochloric acid solution to dissolve any floc. Transfer to a liter volumetric flask and dilute to volume. Use a 10-ml. aliquot.

Leach Liquors.[34] Transfer an acidified aliquot of sample, to contain 0.02-0.10 mg. of silica, to a 25-ml. calibrated test tube. Add 2-3 drops of a saturated solution of 2,6-dinitrophenol as indicator. Bubble ammonia gas into the solution to a light yellow end point or until a small amount of white precipitate appears. Flush out the residual gas in the tube with a small amount of water. Mix thoroughly until the precipitate dissolves and a light yellow color appears. Add 5 ml. of 1:20 hydrochloric acid, dilute to volume, mix, and use an aliquot for the determination of silica by the reduction of the silicomolybdate complex with aminonaphthol sulfonic acid.

Soluble Fluorides.[35] Prepare an aqueous solution of the compound. Take an aliquot to contain approximately 5 mg. of silica in a platinum dish. To this add 1 gram of sodium acetate and 2 ml. of glacial acetic acid. Then add 1-2 grams of aluminum chloride and use as sample for development of the yellow silicomolybdate.

Water.[36] Use 50 ml. of clear sample free from iron and phosphate. Develop the yellow color with ammonium molybdate. Alternatively,[37] develop the blue color by reduction with sulfite. By adjustment of the pH to 2.4-2.7 for development of the phosphomolybate there is no interference by phosphate, tannins, iron, and other substances present in natural and boiler waters.

Condensed Steam.[38] Use the sample without preliminary process-

[34] Allen L. Olsen, Edwin A. Gee, Verda McLendon and Delwin D. Blue, *ibid.* 16, 462-4 (1944).

[35] I. P. Alimarin and V. S. Zverov, *Mikrochemie* 22, 89-100 (1937).

[36] M. C. Schwartz, *Louisiana State Univ. Bull.* 30, No. 14, 46 pp. (1938); *cf.* Harold W Knudson, C. Juday and V. W. Meloche, *Ind. Eng. Chem., Anal. Ed.* 12, 270-3 (1940).

[37] H. Lewis Kahler, *Ind. Eng. Chem., Anal. Ed.* 13, 536-9 (1941).

[38] William E. Bunting, *Ind. Eng. Chem., Anal. Ed.* 16, 612-15 (1944); *cf.* M. Zimmerman, *Die Chemie* 55, 28-30 (1942).

ing. Form the silicomolybdate and add aminonaphthol sulfonic acid as reducing agent to form the molybdenum blue. The yellow silicomolybdate may also be read as such.[39]

Boiler Water. *Dilution may be necessary not only to adjust the silica* content, but to reduce the total alkalinity of the sample if the latter is over 350 ppm. as calcium carbonate. Several types of tannins, such as pyrocatechol and pyrogallol, form yellow molybdate complexes and lead to higher silica results. Pyrogallol gives the more intense color of the two.[40] However, these tannins are not reduced to molybdenum blue in solutions adjusted to a pH of 2.4-2.7.

Phosphate-treated Boiler Water and Hot-process Phosphate Softeners.[41] Filter if necessary and dilute to a suitable silica content. Develop the yellow phosphomolybdate color. Phosphomolybdate is formed in 0.2-1 *N* sulfuric acid with an optimum range of 0.5-0.7 *N*. It will not be reduced at 1.2-1.5 *N*. Silicomolybdate is formed in 0.1-0.25 *N* sulfuric acid and once formed is stable in 3-3.5 *N* sulfuric acid and will be reduced under those conditions. Such adjustments permit estimation of silica in boiler waters without interference by phosphate.[42]

Biological Samples.[43] The silica content varies and there is little correlation between the weight of ash of a biological product and the silica content. Ash a suitable aliquot of sample in a muffle furnace, cool, and acidify with 10 ml. of hot 1:10 hydrochloric acid. Warm gently for 5-10 minutes and cool somewhat. Centrifuge for 15 minutes at 2000 rpm. to bring down silica. Arsenic and phosphate are removed in solution at this point. Dry the residue, mix with an equal amount of sodium carbonate, and fuse. Dissolve in 15 ml of water and make just acid with 1:5 hydrochloric acid. Use as sample for the determination of silica by reduction to molybdenum blue.

Alternatively,[44] add to a 0.5-gram sample in a platinum crucible 1

[39] Frederick G. Straub and Hillary A. Grabowski, *Ind. Eng. Chem., Anal. Ed.* 16, 574-5 (1944).

[40] H. Lewis Kahler, *Ind. Eng. Chem., Anal. Ed.* 13, 536-9 (1941).

[41] M. C. Schwartz, *ibid.* 14, 893-5 (1942).

[42] Yu. M. Kostrikin and E. G. Kochneva, *Izvest. VT* 1 15, No. 11, 25-8 (1946).

[43] Leyton G. Cesar, *Rev. quím. farm.* (Santiago, Chile) No. 35, 1-3 (1945); cf. Alexander O. Gettler and Charles J. Umberger, *Am. J. Clin. Path., Tech. Sect.* 9, 1-3 (1945).

[44] L. Isaacs, *Bull. soc. chim. biol.* 6, 157-68 (1924); cf. Earl J. King, *J. Biol. Chem.* 80, 25-31 (1928); *Biochem. J.* 33, 944-54 (1939); Haruo Sakane, *J. Oriental Med.* 32, 95-8 (1940).

ml. of a saturated boric acid solution, 1 ml. of a 5 per cent calcium nitrate solution, and 2 ml. of concentrated nitric acid. Heat on a water bath until dissolved, then over a free flame until the residue begins to char. Add concentrated nitric acid and heat again until a white ash is obtained. Moisten with 0.5 ml. of concentrated nitric acid and heat to drive off excess acid. Add 3 ml. of water and 3 ml. of a 4 per cent sodium hydroxide solution. Dilute to a known volume and use an aliquot for development of molybdenum blue by sodium sulfite reduction. By suitable use of filters any superimposed yellow color may be eliminated.[45]

Separation as Fluosilicic Acid.[46] In the absence of nitrates, chlorides, and borates the silica may be distilled as fluosilicic acid. The limitation is largely due to corrosion of the still. Transfer the sample, of a size varying with the silica content, to a lead still. For samples of quartz or those otherwise resistant to decomposition, fuse with sodium carbonate in platinum to convert the silica to sodium silicate before transferring to the still. Add excess of sodium fluoride and concentrated sulfuric acid. Distill in the range of 115-140°, absorbing the distillate in water. Dilute the distillate to a known volume and use an aliquot for development of the yellow color with ammonium molybdate.

Separation of Arsenate, Phosphate, and Silicate as Molybdate Complexes. The isolation in nonaqueous solution of molybdenum blue due to silica has been described under arsenic (page 188). Read the solution so obtained against a calibration curve developed under similar conditions.

Standard

Fuse 0.2141 gram of pure anhydrous silica with 2 grams of sodium carbonate in a platinum crucible. Heat at slightly above fusion temperature for 15 minutes, cool, and dissolve the melt in warm water, using a platinum dish as a container. Cool, transfer to a liter volumetric flask, and dilute volumetrically, mixing thoroughly. Store in wax or hard rubber. This corresponds to 0.1 gram of silicon per liter or 0.1 mg per ml.

Silica as the Silicomolybdate

Silicon, like phosphorus and arsenic, reacts to form a yellow molyb-

[45] H. Kaiser and E. Wetzel, *Angew. Chem.* **50**, 865-6 (1937).
[46] T. R. Scott, *J. Council Sci. Ind. Research* **19**, 103-16 (1946).

date complex in weakly acid solutions.[47, 48] This yellow is commonly referred to as silicomolybdate yellow, but also sometimes as molybdisilicic acid. At low acid concentrations the reaction with silica is more sensitive than with phosphate.[49] The yellow color is believed to be a complex with the composition $H_8Si(Mo_2O_7)_6 \cdot H_2O$.[50] Thus the mol ratio is one of silica to 12 of molybdic oxide,[51] and only a slight excess of molybdate is necessary for completion of the reaction.

The molybdate reaction is generally carried out at room temperature. Hydrolysis of the yellow compound with mineral acid is prevented by buffering with acetates. The effect of iron is overcome by addition of an excess of phosphoric acid which will destroy the color of the ferric ion and not affect the preformed silicomolybdate. Addition of aluminum ion eliminates interference by fluorides.

At very low silica concentrations the color development is very critical. Dilution of the sample of higher concentration makes the method more sensitive. Increasing the time for color development has little effect on solutions with low silica concentrations and broadens the permissible pH range for solutions with higher concentrations of silica.

For general application it must be borne in mind that silica undergoes polymerization and colloidal change in solutions that are about neutral. Care must be taken that silicon is not converted to colloidal silicic acid, causing low results.[52] Some degrees of aggregation of polymerized silica do not form the yellow molybdate complex. In such cases, complete removal of foreign ions and adjustment of the colloidal particle size permit the determination of silica.[53] Alkaline decomposition of samples converts silica to a soluble noncolloidal form.[54]

Since phosphorus, arsenic, and germanium form similar complexes with molybdenum,[55] they can interfere in the determination of silica. The

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[47] A. Jolles and F. Neurath, *Z. angew. Chem.* **11**, 315 (1898); F. Dienert and F. Wandenbulcke, *Compt. rend.* **176**, 1478-80 (1923).

[48] This reaction is discussed in more detail in the chapter on phosphorus. It is suggested that the method there be consulted for further details.

[49] T. S. Harrison and H. Storr, *J. Soc. Chem. Ind.* **63**, 154-7 (1944).

[50] A. I. Kryagova, *J. Gen. Chem.* (U.S.S.R.) **8**, 625-34 (1938); cf. I. P. Alimarin and V. S. Zverev, *Mikrochemie* **22**, 89-100 (1937).

[51] Harold W. Knudson, C. Juday and V. Meloche, *Ind. Eng. Chem., Anal. Ed.* **12**, 270-3 (1940).

[52] B. Visintin and N. Gandolfo, *Ann. chim. applicata* **31**, 509-22 (1941).

[53] M. F. Adams, *Ind. Eng. Chem., Anal. Ed.* **17**, 542-3 (1945).

[54] J. A. Brabson, I. W. Harvey, G. E. Maxwell and O. A. Schaeffer, *ibid.* **16**, 705-7 (1944).

[55] Paul Krumholz, *Z. anorg. allgem. Chem.* **212**, 91-6 (1933).

arsenic complex does not form at room temperature. Interference from phosphates has been prevented by various means. These include precipitation with magnesia mixture, calcium chloride, calcium chloride and calcium carbonate, calcium chloride and ammonium hydroxide, or calcium chloride and a sodium borate-sodium hydroxide mixture. By adjusting the pH, interference from phosphates is prevented without separation.[56] Another method of treatment involves the destruction of the preformed phosphoric acid complex by the addition of citric,[57], oxalic,[57, 58, 59] or tartaric[59] acids, or ammonium tartrate[60] or sodium citrate.[61] One mol of molybdic oxide to 1 of oxalic acid is required.[62]

Color comparison of the yellow molybdate complex may be made with permanent potassium chromate standards.[63] Calcium chromate is also suitable.[64] It follows that the method is subject to chromium interference in direct proportion to the amount. By obtaining a basic calibration curve from a sample containing no chromium, correction for the chromium content is possible up to 0.80 per cent of silicon.[65] The precision of the method thus used is ±0.02 per cent silicon. Picric acid solutions[66] are also suitable standards, provided aluminum is removed with disodium phosphate. Beer's law holds for photometric determination at 410 mμ and above. Light filters may be prepared by applying a 5 per cent gelatin solution containing 0.02 per cent picric acid solution to glass.[67] In general, for small amounts colorimetric methods are at least as accurate as gravimetric. Several variations of the procedure must be considered to take care of variations in the other ions present.

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[56] H. L. Kahler, *Ind. Eng. Chem., Anal. Ed.* 13, 536-9 (1941).

[57] M. C. Schwartz, *ibid.* 6, 364-7 (1934); *ibid.* 14, 893-5 (1942); O. P. Case, *ibid.* 16, 309-11 (1944).

[58] William E. Bunting, *ibid.* 16, 612-15 (1944).

[59] I. P. Alimarin and V. S. Zverev, *Mikrochemie* 22, 89-100 (1937).

[60] A. J. Boyle and V. V. Hughey, *Ind. Eng. Chem., Anal. Ed.* 15, 618-19 (1943).

[61] F. K. Lindsay and R. G. Bielenberg, *ibid.* 12, 460-3 (1940).

[62] Ernst Tschopp and Emilio Tschopp, *Helv. Chim. Acta* 15, 793-809 (1932).

[63] H. W. Swank and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 6, 348-50 (1934); M. C. Schwartz, *ibid.* 14, 893-5 (1943).

[64] Hans Pinsl, *Z. Metallkunde* 27, 107-14 (1935).

[65] David Rozental and Hallock C. Campbell, *Ind. Eng. Chem., Anal. Ed.* 17, 222-4 (1945).

[66] Earl J. King and C. A. Lucas, *J. Am. Chem. Soc.* 50, 2395-7 (1928); K. M. Popov and M. L. Vegrin, *Zavodskaya Lab.* 6, 502-3 (1937); H. A. J. Pieters and C. Popelier, *Chem. Weekblad* 40, 2-10 (1943).

[67] F. Ya. Galakhov, *Zavodskaya Lab.* 10, 90-2 (1941).

Procedure. *Reducing Agents Present.* Transfer a sample containing less than 1 mg. of silicon to a 100-ml. volumetric flask. Add 5 ml. of 8 per cent ammonium molybdate solution. Reducing agents will cause a green color to develop. Add 0.1 gram of potassium persulfate as oxidizing agent. A yellow color will then develop within 5 minutes. Read the transmittance at 420 mμ, compare with a curve, and correct for a reagent blank.

Phosphate Present. To an aliquot of sample containing up to 1 mg. of silicon, add 5 ml. of 10 per cent ammonium molybdate solution. Dilute to about 80 ml. and allow to stand for 10 minutes, after which add 5 ml. of 5 per cent citric acid solution. Dilute to 100 ml., mix thoroughly, and read the transmittance immediately with a filter centering at 410 mμ. Alternatively, compare with standards.

Phosphate and Iron Present. Dilute an aliquot containing 0.1-0.5 mg. of silicon to about 25 ml. and add 5 ml. of 10 per cent ammonium molybdate solution. Allow 6 minutes to elapse to develop the maximum intensity and add 10 ml. of 2 per cent sodium fluoride solution to decolorize the tint due to phosphate and iron.[68] Mix well, dilute to 50 ml., and read immediately at 420 mμ. As a blank take another portion of sample, add 5 ml. of water and 10 ml. of the 2 per cent sodium fluoride solution, dilute to 50 ml. and read. Subtract the reading of this blank.

Silica as Molybdenum Blue

The reduction of preformed yellow silicomolybdate complex gives the molybdenum blue, parallel to the most common method for determination of phosphorus.[69] This indirect method is the most sensitive method for estimation of silica. A suitable pH for formation of the silicomolybdic acid is 1.6. The minimum transmittance of the molybdenum blue obtained on reduction is at 820 mμ.[70] The color is stable for 12 hours and conforms to Beer's law at that band. Various other levels are also used for reading. There are numerous methods of reduction. Those of major importance use 1,2,4-aminonaphthol sul-

[68] Robert Weihrich and Walter Schwartz, *Arch. Eisenhüttenw.* 14, 501-3 (1941).

[69] This reaction is discussed in more detail in the chapter on phosphorus. It is suggested that the method there be consulted for further details.

[70] D. F. Boltz and M. G. Mellon, *Anal. Chem.* 19, 873-7 (1947).

fonic acid,[71] sodium sulfite,[72] stannous chloride,[73] hydroquinone,[74] which is the original Bell and Doisy reagent for phosphorus, hydroxylamine,[75] p-hydroxyphenylglycine,[76] and ferrous ion in the presence of oxalic acid.[77] Some of secondary importance are sodium hyposulfite, α-naphthol,[78] etc.

The reduction with 1,2,4-aminonaphthol sulfonic acid is very sensitive.[79] The time factor is not as critical as when sodium sulfite is used as reducing agent. By use of oxalic acid, interference from phosphates may be eliminated. Citric acid eliminates interference by calcium. Aluminum, zinc, and iron remain in solution as complexes by the use of ammonium tartrate. Reading of the molybdenum blue complex at a pH of 7.2 is possible. Phosphate ions do not interfere when the pH is adjusted to 4.2-6.8.[80] The reaction is demonstrably more sensitive than that by the yellow silicomolybdate. Hence it is applied to 0.001-0.2 mg. of silica per 100 ml., but if the concentration in the sample exceeds that level it is desirable to avoid use of that reducing agent.

When sodium sulfite is used as a reductant, the permissible range of concentration is to about 7 times higher. The color with sulfite reduction conforms to Beer's law only after 48 hours.[81] Thus the time which elapses between the addition of the ammonium molybdate reagent and of sodium sulfite reagent and the reading has a marked effect on the reading.[82] The transmittance of the solution decreases as the time after addition of sodium sulfite increases. The precision of reading under optimum concentrations with this reagent is ±4 per cent.[83] The presence

[71] Frederick G. Straub and Hilary A. Grabowski, *Ind. Eng. Chem., Anal. Ed.* 16, 574-5 (1944); William E. Bunting, *ibid.* 16, 612-15 (1944).

[72] J. H. Foulger, *J. Am. Chem. Soc.* 49, 429-35 (1927); H. Lewis Kahler, *Ind. Eng. Chem., Anal. Ed.* 13, 536 9 (1941); J. T. Woods and M. G. Mellon, *ibid.* 13, 760-4 (1941); A. J. Boyle and V. V. Hughey, *ibid.* 15, 618 19 (1943); J. A. Brabson, I. W. Harvey, G. E. Maxwell and O. A. Schaeffer, *ibid.* 16, 705-7 (1944).

[73] Donald F. Clausen and Harold D. Roussopoulas, *Anachem News* 6, 41-4 (1946).

[74] A. Mayrhoffer, A. Wasitzky and W. Korn, *Mikrochemie* 20, 29-48 (1936).

[75] Walter Parri and Giuseppe Scotti, *J. pharm. chim.* 18, 513-27 (1933).

[76] Floyd De Eds and C. W. Eddy, *J. Biol. Chem.* 114, 667-72 (1936).

[77] C. H. R. Gentry and L. G. Sherrington, *J. Soc. Chem. Ind.* 65, 90-2 (1946).

[78] Leyton G. Cesar, *Rev. quím. farm.* (Santiago, Chile) No. 35, 1-3 (1945).

[79] Allen L. Olsen, Edwin A. Gee, Verda McLendon and Delwin D. Blue, *Ind. Eng. Chem., Anal. Ed.* 16, 462-4 (1944).

[80] Walter Parri, *Ann. chim. applicata* 33, 193-204 (1943).

[81] J. T. Woods and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 13, 760-4 (1941).

[82] Frederick G. Straub and Hilary A. Grabowski, *ibid.* 16, 574-5 (1944).

[83] J. A. Brabson, I. W. Harvey, G. E. Maxwell and O. A. Schaeffer, *ibid.* 16, 705-7 (1944).

of 400 times as much aluminum as of silica is permissible in the molybdenum blue reduction. If hydroquinone is used as the reducing agent, calcium ions must be eliminated by the addition of citric acid.[84]

Reduction of the yellow silicomolybdate to molybdenum blue by ferrous ions in the presence of oxalic acid[85] depends on the oxalic acid lowering the oxidation potential of the ferric-ferrous system and thus preventing interference from phosphorus, arsenic, and vanadium. It has the advantage over stannous chloride because of its greater stability. Variations in the excess of ferrous ammonium sulfate used have no effect. The maximum color intensity is reached in 30 seconds and the color is stable for at least 24 hours under optimum conditions. It is desirable to standardize on a definite interval because decrease of color with time sometimes occurs.

Stannous chloride is a desirable reagent for determination of silicon in steel, although even then accuracy only to 8 per cent is obtained.[86] The same reagent, applied to magnesium containing 0.015-0.05 per cent of silicon, gave similar accuracy.[87] Permanent standards for the molybdenum blue complex are prepared from ammoniacal solutions of copper sulfate containing a very small quantity of picric acid.[88]

Procedure. *Reduction with 1,2,4-Aminonaphthol Sulfonic Acid.* To prepare the necessary reagent, dissolve 75 grams of ammonium molybdate in water to which have been added 322 ml. of 1:9 sulfuric acid, and dilute to 1 liter. As sample take 0.001-0.01 mg. of silica and dilute to about 50 ml. Add 1 ml. of the reagent. If phosphate ions are present, add after 5 minutes 4 ml. of 10 per cent tartaric acid and mix. Citric and oxalic acids are also used for the same purpose. Prepare a reducing agent by dissolving 7 grams of anhydrous sodium sulfite in 100 ml. of water and by adding 1.5 grams of 1,2,4-aminonaphthol sulfonic acid. Dissolve and add a solution of 90 grams of sodium bisulfite in 800 ml. of water. Mix and dilute to 1 liter. Reduce the sample solution with 1 ml. of this reagent and dilute to 100 ml. After 1 minute read the transmittance at 700 mμ or compare with standards. For higher concentrations, allow a longer period for full-color development before taking the reading.

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[84] H. A. J. Pieters and C. Popelier, *Chem. Weekblad* 40, 2-10 (1943).
[85] C. H. R. Gentry and L. G. Sherrington, *J. Soc. Chem. Ind.* 65, 90-2 (1946).
[86] Donald F Clausen and Harold D. Roussopoulas, *Anachem News* 6, 41-4 (1946).
[87] A. L. Davydov and O. A. Malinovskaya, *Zavodskaya Lab.* 9, 964-7 (1940).
[88] A. Mayrhoffer, A. Wasitzky and W. Korn, *Mikrochemie* 20, 29-48 (1936).

Reduction with Stannous Chloride.[89] To a sample containing 0.1-0.01 mg. of silicon in slightly acid solution add 2 ml. of a 5 per cent solution of ammonium molybdate. Mix and let stand until the yellow silicomolybdate complex has formed, usually about 7 minutes. Add 6 ml. of 1:1 sulfuric acid and enough water to dilute to about 90 ml. Add 2 ml. of fresh 0.5 per cent stannous chloride solution dropwise with mixing and dilute to 100 ml. Read the transmittance photometrically as soon as the maximum intensity of color has developed or compare with a standard prepared at the same time.

Reduction with Sodium Sulfite. Make an aliquot of sample containing 0.025-0.25 mg. of silica just acid to thymol blue indicator. The pH is 1.0-1.4. Add with stirring 5 ml. of 1:9 hydrochloric acid, 5 ml. of 1:2 acetic acid and 5 ml. of 10 per cent ammonium molybdate solution. Stir vigorously for 1 minute and wait 5 minutes for the development of silicomolybdate. Transfer to a 250-ml. flask and add from a pipet, with vigorous shaking, 20 ml. of 17 per cent sodium sulfite solution. Eight minutes later add 5.0 ml. of 1:2 acetic acid, dilute to the mark, and mix thoroughly. After 30 minutes, using a 650-mμ filter, determine the color by comparison with a calibration curve, using water as reference standard. If aluminum salts are present in large concentrations, prepare two calibration curves based on 0.2 and 0.4 gram of alumina in the sample. If phosphorus is present, the tendency is toward high results.

Alternatively, to a similar aliquot which need not have had the pH adjusted but should be just acid, add 20 ml. of water, 6 ml. of 10 per cent acetic acid, and 10 ml. of 10 per cent ammonium molybdate solution. Heat on a steam bath for 5 minutes and add 4 ml. of saturated sodium sulfite solution. Mix, cool, and dilute to 250 ml. Determine silicon photometrically or by comparison with a standard prepared at the same time.

Reduction with Hydroquinone. Neutralize an aliquot containing 0.005-0.05 mg. of silica with 1:35 sulfuric acid. Add, with thorough mixing after each addition, 2 ml. of 5 per cent ammonium molybdate solution in 1:35 sulfuric acid, 10 ml. of 1:5 sulfuric acid, and 2 ml. of 5 per cent hydroquinone solution. The sulfuric acid concentration eliminates interference from phosphorus. Dilute to 50 ml. and read the transmittance after 20 minutes.

[89] A. A. Tikhonova, *Zavodskaya Lab.* **11**, 616-17 (1945).

Reduction with Ferrous Ammonium Sulfate. To a 25-ml. aliquot of sample to contain 0.008-0.26 mg. of silicon, add 10 ml. of 2.5 per cent ammonium molybdate, mix, and let stand for 5 minutes. Add 10 ml. of 4 per cent oxalic acid, mix, and add 5 ml. of 6 per cent ferrous ammonium sulfate solution. Read with a 608-mμ filter. To a second aliquot of equal volume as blank, add, with mixing after each addition, 10 ml. of 4 per cent oxalic acid, 10 ml. of 2.5 per cent ammonium molybdate, and 5 ml. of 6 per cent ferrous ammonium sulfate solution.

Miscellaneous

Limestone and dolomite solutions are analyzed by a spot test [90] by decomposing 0.01-5 grams of sample in platinum with an excess of concentrated nitric acid. Filter the residue, wash, dry, and fuse with twice the quantity of sodium carbonate. Cool, add water to the melt, and boil. To the filter paper add a drop of 10 per cent ammonium molybdate reagent and a drop of solution. Dry gently over a flame and add another drop of molybdate solution. Dry again, add a drop of benzidine acetate solution prepared by saturating 1:1 acetic acid with sodium acetate and then with benzidine. Compare with simultaneously prepared standards.

A nephelometric comparison under reflected light is possible by mixing a washed silica precipitate with dilute glycerol until fine particles are no longer noticeable.[91] Silicic acid gives a deep blue unstable coloration in the presence of pyrrol,[92] which in phosphoric acid is sensitive to 2 ppm. Iron increases the sensitivity of the reaction.

Silica is also estimated by the opalescence with a cocaine-ammonium molybdate reagent.[93] As reagent, mix 4.3 ml. of 5 per cent sodium molybdate solution, 4.3 ml. of 2.5 per cent cocaine hydrochloride solution, and 11.4 ml. of 96 per cent acetic acid. When 4 volumes of this are added to 6 volumes of sample, the turbidity appears in 2-3 minutes and will detect 0.0005 mg. of silicon

[90] N. A. Tananaev and A. M. Shapovalenko, *J. Applied Chem.* (U.S.S.R.) 11, 352-4 (1938).

[91] N. S. Nikola'ev, *Zavodskaya Lab.* 10, 536 a (1941).

[92] R. Berg and M. Teitelbaum, *Mikrochem., Emich Festschr.* 1930, 23-6.

[93] H. Wachsmuth, *J. pharm. Belg.* 19, 575-7, 593-5, 609-13, 627-31 (1937).

CHAPTER 48

BORON

Boron is one of the relatively rare elements. Occurrence is in the form of borates and boric acid in nature and as borides in alloys. The first two are important in minerals, soils, plant tissue, etc. A few thousandths of a per cent of boride is important in steel. Boron as the borate forms a volatile ester with methanol, methyl borate being a means of isolation of boron. For this determination, Pyrex glass must be avoided because of its boron content.

The various methods of determination are uniformly by complex reactions with organic materials, and they may be either colorimetric or fluorimetric. No single method is sufficiently important to be outstanding, but the method using quinalizarin is most used. Numerous alternatives are available.

Samples

Carbon Steel. *Acid-soluble Boron.*[1] If the steel contains up to 0.002 per cent of boron use a 0.5-gram sample, up to 0.004 per cent of boron use a 0.25-gram sample, and up to 0.008 per cent use a 0.10-gram sample. Transfer to a 100-ml. quartz flask A, and to a similar flask B add 50 ml. of absolute methanol and 5 ml. of a 0.56 per cent calcium hydroxide suspension. To the trap C add sufficient 0.56 per cent calcium hydroxide suspension to form a liquid seal. Add 10 ml. of 85 per cent orthophosphoric acid to the flask containing the sample, connect the apparatus as shown in Figure 23 and heat flask A gently until reaction ceases. Remove the source of heat from flask A. Place flask B in a water bath and heat until 25 ml. of methanol have distilled into flask A. Disconnect the water supply from condenser D, place both flasks in water baths, and heat so that methanol will reflux evenly between flasks A and B.

Remove the flasks from the water baths and transfer the solution from flask B and from trap C to a porcelain casserole, washing the apparatus with water, then with 2 drops of 1:9 hydrochloric acid, and again with water. Add the washings to the sample solution and evapo-

[1] Proposed ASTM method.

rate to dryness on a steam bath. Remove, cool to room temperature, and use as sample for the determination of acid-soluble boron by the curcumin method.

Acid-insoluble Boron. Follow the preparation of sample for acid-soluble boron. After the methanol has refluxed between A and B, remove the flasks from the water bath and dilute the solution in flask A to 90 ml. with 1:8 hydrochloric acid. Filter through ashless filter paper containing a small amount of filter pulp and wash the flask thoroughly with hot 1:50 hydrochloric acid. Wash the residue on the filter with hot 1:50 hydrochloric acid, followed by cold water to remove free acid. Transfer paper and residue to a platinum crucible, add 5 ml. of a 0.56 per cent calcium hydroxide suspension, and evaporate to dryness. When dry, ignite at 600-700° until the carbon is completely burned out. Add about a gram of sodium carbonate and fuse this with the residue, tilting the crucible so that the fused mass forms a ball. Cool, apply a slight pressure on the crucible wall to loosen the fused mass, and transfer to flask A. Place the flask under cold running water to bring the temperature to 10-15°. Add 4 ml. of 85 per cent orthophosphoric acid to the crucible in which the fusion was made, warm to dissolve the remainder of the fusion, cool, and add to flask A containing the bulk of the fusion. Rinse the crucible with two 3-ml. portions of 85 per cent orthophosphoric acid and add to flask A. Connect the flask with the flask B containing methanol and the trap C containing the 0.56 per cent suspension of calcium hydroxide. Heat the flask A to obtain a complete solution of the fusion and continue

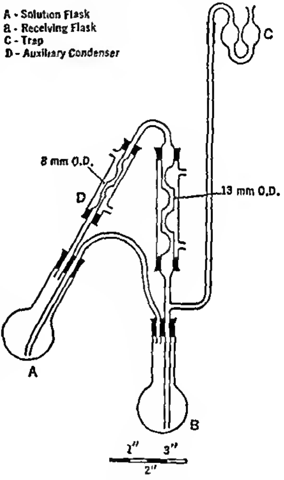

FIG. 23
Apparatus for the Determination of Boron in Steel

as outlined for the determination of acid-soluble boron (page 700), beginning "Remove the source of heat from flask A."

Total Boron. Add the percentage of acid-soluble boron to the percentage of acid-insoluble boron. The boron-containing sample may also be refluxed with 1:4 sulfuric acid until decomposed, carbides decomposed with hydrogen peroxide, and then boron distilled as methyl borate.[2]

Soils. *Available Boron.*[3] Transfer a 20-gram sample of air-dried, 20-mesh material to a boron-free round-bottom flask. Add 40 ml. of distilled water and attach a reflux condenser. Boil for 5 minutes, disconnect the condenser, and stopper the flask. Cool the contents of the flask and filter with suction or centrifuge at 2000 rpm. until the supernatant liquid is clear. To help clarify the solution, add not more than 0.05 gram of calcium chloride dihydrate. Transfer a 20-ml. aliquot to a platinum dish and add 5 drops of 40 per cent potassium carbonate solution. If a porcelain crucible is used, substitute 2 ml. of a saturated calcium hydroxide solution for potassium carbonate. Evaporate to dryness and ignite gently to destroy nitrates and organic matter. Cool, add 5 ml. of 1:100 sulfuric acid, and mix thoroughly with the residue. Use 1-ml. aliquots of this solution for determination of boron by the quinalizarin method.

Total Boron. Fuse 0.5 gram of sample in a platinum crucible with 3 grams of anhydrous sodium carbonate. Cool and place the crucible in a 250-ml. beaker containing 50 ml. of water. Place a cover glass on the beaker and add 1:10 sulfuric acid at 3-minute intervals until the melt disintegrates and the pH of the solution is 5.5-6.0. Transfer the solution to a 500-ml. volumetric flask. Wash the beaker and flask several times with water and add the washings to the flask, keeping the volume within 150 ml. Dilute to volume with methanol or ethanol and mix the contents thoroughly. Filter with suction or centrifuge until the supernatant liquid is clear.

To a 400-ml. aliquot of clear solution add 100-150 ml. of water to prevent subsequent precipitation. Add a 40 per cent solution of potassium carbonate until the solution is alkaline and evaporate to 10-15 ml. Transfer to a platinum dish, evaporate to dryness, and ignite to destroy

[2] B. A. Generozov, *Zavodskaya Lab.* **12**, 25-30 (1946).

[3] K. C. Berger and E. Truog, *Ind. Eng. Chem., Anal. Ed.* **11**, 540-5 (1939).

nitrates and organic matter. As an alternative method for the removal of organic matter, evaporate the sample gently to dryness, take up in a 1:9 mixture of 85 per cent orthophosphoric acid and absolute methyl alcohol, and distill to obtain boron as the methyl ester of boric acid. The presence of a small amount of uncombined water prevents the complete distillation of the boron. Cool and dissolve the residue in 4 ml. of 1:100 sulfuric acid. Use 1-ml. aliquots of this solution for the estimation of boron by the quinalizarin method.

Plants. *Total Boron.*[4] Transfer 0.25-0.50 gram of oven-dried 20-mesh sample to a platinum crucible. Ignite to a white or gray ash. It is not necessary to add an alkali to plant tissue to prevent loss of boron on heating, since sufficient basic material is released from organic combination on ignition to hold boron as nonvolatile borate. Cool, add 5 ml. of 1:100 sulfuric acid, and mix thoroughly with the ash. Allow to settle and use a 1-ml. aliquot of the supernatant liquid for the determination of boron by the quinalizarin method.

Standard

Dissolve 0.5716 gram of boric acid in water and dilute to 1 liter. This contains 0.1 mg. of boron per ml.

Boron by Quinalizarin

The addition of boric acid to a polyhydroxy aromatic compound[5] in reasonably concentrated sulfuric acid causes a definite color change which may be used to estimate the amount of boron present. The change is due to the formation of a chelate ring. The most sensitive is 1,2,5,8-tetrahydroxyanthraquinone,[6] quinalizarin, which changes from a pink color to a bluish hue with increasing concentrations of boron. The presence of sulfuric acid aids in splitting off water and prevents the reaction from reversing. Maximum absorption occurs at 600 mμ. Other related reactions are those with purpurin, alizarin S,[7] Chromotrop

[4] K. C. Berger and E. Truog, *Ind. Eng. Chem., Anal. Ed.* 11, 540-5 (1939).

[5] F. Feigl and P. Krumholz, *Mikrochem. Pregl Festschr.* 77-86 (1929).

[6] K. Scharrer and R. Gottschall, *Z. Pflanzenernähr., Düngung Bodenk.* 39, 178-97 (1935); K. C. Berger and E. Truog, *Ind. Eng. Chem., Anal. Ed.* 11, 540-5 (1939); G. A. Rudolph and L. C. Flickinger, *Steel* 112, No. 14, 114 (1943); S. Weinberg, K. L. Proctor, and O. Milner, *Ind. Eng. Chem., Anal. Ed.* 17, 419-22 (1945).

[7] Denis Dickinson, *Analyst* 68, 106-9 (1943).

2B,[8] 1-amino-4-hydroxyanthraquinone,[9] and resacetophenone.[10] Aside from those cited requiring concentrated acid there is one with flavonols.[11]

The method as given is for quinalizarin whose curve does not conform exactly to Beer's law so that points on the standard curve must be determined at small intervals over the working range. Slight variations in the concentration of sulfuric acid in the sample solution have a marked effect on the intensity of color developed. At least 44 per cent of sulfuric acid by weight must be present in order to produce a visible color change due to boron.[12] The sensitivity increases as the acid approaches a concentration of 93 per cent by weight of sulfuric acid, and 98 per cent may be necessary for full color development.[13] The sensitivity decreases rapidly as the temperature rises above room temperature. The method is sensitive to 0.0001 mg. of boron.

Boron is freed from organic matter by adding potassium or sodium carbonate or calcium hydroxide to the sample, evaporating and igniting gently. If interfering ions are present, evaporate the sample to dryness, take up in a 1:9 mixture of 85 per cent orthophosphoric acid and absolute methanol, and separate boron by distillation as the methyl ester of boric acid.[14]

Fluorides in excess of 500 ppm. of water-soluble fluorides, or over 5000 ppm. of total fluorides, nitrates, dichromates, and other oxidizing agents interfere by decolorizing the solution. Fluorides are removed by precipitation with thorium chloride. Nitrates are removed by ignition. Germanic acid is the only substance that undergoes a similar color change.[15] To produce a given color change, however, requires 200 times as much germanium as boron.

Procedure. To a 1-ml. aliquot of this solution add 9 ml. of 98.5 per cent sulfuric acid, stopper, and cool. Add 0.5 ml. of quinalizarin solution containing 0.01 gram of quinalizarin in 100 ml. of 88.65 per cent sulfuric acid. Mix thoroughly by gently whirling, allow to stand at least 15 minutes, and compare with standards or with a calibration curve.

[8] A. Stettenbacker, *Mitt. Lebens. Hyg.* 34, No. 1/2, 90-7 (1943).

[9] J. A. Radley, *Analyst* 69, 47 (1944).

[10] K. Neelakantam and L. R. Row, *Proc. Indian Acad. Sci.* 16a, 349-58 (1942).

[11] K. Tauboeck, *Naturwissenshaften* 30, 439 (1945).

[12] G. Stanley Smith, *Analyst* 60, 735-9 (1935).

[13] J. S. McHargue and P. N. Scripture, *J. Assoc. Official Agr. Chem.* 28, 797-9 (1945).

[14] E. C. Owen, *Analyst* 71, 210-17 (1946).

[15] N. S. Poluektov, *Mikrochemie* 18, 48-9 (1935); L. Szebellédy and St. Tanay, *Z. anal. Chem.* 107, 26 30 (1936).

If the boron content is very high, it may be necessary to lower the acidity so as to require a larger amount of boron to effect a given change in color intensity.

Boron by Curcumin

In acetone solution curcumin forms a colored complex with boron, the color ranging from yellow-green to deep rose, depending on the boron concentration.[16] The color is read with a 540-mμ green filter. The boron content of the filter glass should be taken into account when calculating the boron present in the sample.

Procedure. This method is designed to take an alkaline residue in which boron has been concentrated as the borate. To this residue which should contain 0.001-0.01 mg. of boron, add 1 ml. of 1:4 hydrochloric acid and 5 ml. of an oxalic acid solution prepared by dissolving 50 grams of oxalic acid in 450 ml. of acetone and filtering. Mix and add 2 ml. of curcumin solution containing 0.1 gram of curcumin dissolved in 400 ml. of 95 per cent ethanol. When the residue in the casserole has dissolved, evaporate to dryness on a steam bath at $55° \pm 3°$. To the residue in the cooled casserole add 25 ml. of acetone. Filter the dissolved residue through a fritted glass crucible into a 100-ml. volumetric flask. Wash the crucible and contents with six 4-ml. portions of acetone. Dilute the contents of the flask to volume, mix well, and measure the transmittance of an aliquot portion using a 540-mμ green filter. Compare with standards or with a calibration curve.

Boron Fluorimetrically by Benzoin

Addition of benzoin in slightly alkaline medium to a boron solution gives a greenish white fluorescent solution which is a linear function of boron concentration up to 0.01 mg. of boron in 50 ml.[17] Benzoin is $C_6H_4COCHOHC_6H_4$, which has also been used for zinc. The reaction is also given with antimony and beryllium, which, as well as zinc, must therefore be absent for its application to boron. The method will detect 0.0002 mg. of boron. Visual comparison will show the difference between 0.0002, 0.0005, and 0.0008 mg. in 10 ml.[18]

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[16] Proposed ASTM method.

[17] Charles E. White and M. H. Neustadt, *Ind. Eng. Chem., Anal. Ed.* 15, 599 (1943); Charles E. White, Alfred Weissler, and David Busker, *Anal. Chem.* 19, 802-5 (1947).

[18] M. H. Fletcher, C. E. White, and M. S. Sheftel, *Ind. Eng. Chem., Anal. Ed.* 18, 179 (1946).

Procedure. Transfer a sample containing about 0.01 mg. of boron in 1 ml. of solution to a 50-ml. volumetric flask. Add 1 ml. of 2.4 per cent sodium hydroxide solution and dilute to about 45 ml. with 95 per cent ethanol. Add 4 ml. of 0.5 per cent solution of benzoin in ethanol and dilute to volume with ethanol. Read the fluorescence when irradiated with 440-630 mμ. Turbidity does not interfere. Take the maximum, which is reached in about 4 minutes, as the reading and compare with a standard curve.

Miscellaneous

The turmeric paper test may be used to determine small amounts of boron.[19] Some features of convenience of this reaction are more than offset by the lengthy procedure, whether by turmeric paper or solution.

A very roundabout indirect method is applicable to boron.[20] In brief the colorless stable BF_4^- complex is formed in solution neutral to phenolphthalein by reaction with a known excess of fluoride ion. A mixture of ferric chloride and sulfosalicylic acid is prepared and added. This is decolorized in proportion to the excess of fluoride ion, decreasing the added red color. Reading of the color photometrically permits calculation of the excess fluoride ion and therefore indirectly of the boron content.

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[19] Gabriel Bertrand and H. Agulhon, *Compt. rend.* 157, 1433-6 (1913); K. A. Kar, *Metals and Alloys* 9, 175-7 (1938); A. R. C. Haas, *Plant Physiol.* 20, 323-43 (1945).

[20] D. Monnier, Y. Rusconi, and P. Wenger, *Anal. Chim. Acta* 1, No. 1, 13-18 (1947).

CHAPTER 49

CHLORINE AND CHLORAMINE

Free chlorine is present in water supplies being treated for reduction of bacterial count. It may be present in the air in the vicinity of plants during chlorinations and, although the toxic level is not low in practical terms, it often requires control. Chloramine is an alternative to chlorine for water treatment.

The classical method for chlorine and chloramine is that of the American Public Health Association with *o-tolidine*. Others are given to use as confirmations in special investigations of chlorine contamination.

Samples

Air. Arrange a test tube with a 2-holed rubber stopper fitted with a long entrance tube ending in a capillary extending beneath the surface of the liquid, and a short exit tube. Connect the short exit tube to a tube entering a large bottle. Fit this with a 2-holed rubber stopper, containing as a second tube, a long exit tube extending nearly to the bottom of the bottle. Fill the bottle with water to serve as a siphon and measure the volume of water siphoned off to estimate the volume of air drawn through the solution of reagent, of which 10 ml., preferably o-tolidine, are introduced into the test tube. Aspirate 50-100 ml. of air at moderate speed through the solution of reagent. For comparison, dilute to a suitable volume.

Water. In the absence of interfering substances use the sample as received.

Interfering Substances Present. Boil 100 ml. of the chlorinated sample down to about 75 ml. Cool and dilute to volume. Determine the chlorine value of the interfering substance and subtract from the value obtained on the same sample which was not boiled. Another method is to sample before and after chlorination. Shake the unchlorinated sample with oxygen. Determine the chlorine value of the interfering substance and subtract from the total value obtained after chlorination.

Sewage. To 20 ml. of sample add 5 ml. of concentrated hydrochloric acid and use for determination of chlorine by *o*-tolidine.

Standard

A standard solution of chlorine is not satisfactory as the results are irreproducible unless great care is used. Several manufacturers offer kits with permanently sealed standards for field and factory use. In the methods which follow an artificial standard is given in each case.

Chlorine by o-Tolidine

In the estimation of free chlorine, *o*-tolidine in acid solution produces a yellow color which may be estimated when only 0.01 ppm. of chlorine is present. The usual application is to chlorination of water.[1] The color is stable for only a comparatively short time. At the end of an hour it has faded about 50 per cent. The action is an oxidation-reduction, so that oxidizing agents, reducible substances, and unstable chlorine addition-products behave similarly. Ferric salts produce some color on standing, but for practical purposes they do not have to be considered. Manganese present as manganic hydroxide gives the same reaction as chlorine, 0.05 and 2.0 ppm. of the former giving a color equivalent to 0.03 and 0.5 ppm. of free chlorine.

Procedure. Use the *o*-tolidine reagent prepared as described under manganese (page 398). If less than 1.0 ppm. of chlorine is present in the sample, use 1 ml. of reagent per 100 ml. of sample, mix well, and compare with permanent standards in 5 minutes (Table 11). If more than 1.0 ppm. is present, add 5 ml. of reagent per 100 ml. of sample, mix well, and compare with permanent standards in 15 minutes (Table 11). For high chlorine concentrations allow 30 minutes before making comparison.

Artificial Standard. Prepare a solution of 1.5 grams of crystallized copper sulfate and 1.0 ml. of concentrated sulfuric acid in distilled water and dilute to 100 ml. Dissolve 0.25 gram of potassium bichromate and 1.0 ml. of concentrated sulfuric acid in distilled water and dilute to 100 ml. For accuracy in measurement for standards up to 0.1 ppm. of chlorine each of these should be diluted to 0.1 strength with distilled

[1] American Public Health Association, "Standard Methods for the Examination of Water and Sewage." Ninth Edition, pp. 93-103 (1946).

TABLE 11. STANDARDS FOR CHLORINE WITH O-TOLIDINE

	Volume of Standards to be Diluted to 100 ml.	
Chlorine (ppm.)	*Bichromate Solution (ml.)*	*Copper Sulfate Solution (ml.)*
0.01	0.18	0.3
0.02	0.32	0.5
0.04	0.61	1.0
0.06	0.87	1.4
0.08	1.1	1.7
0.10	1.3	1.9
0.15	1.7	1.9
0.20	2.1	2.0
0.25	2.6	2.0
0.30	3.0	2.0
0.35	3.4	2.0
0.40	3.8	2.0
0.50	4.7	2.0
0.60	5.5	2.0
0.70	6.4	2.0
0.80	7.2	2.0
0.90	8.1	2.0
1.00	9.0	2.0

Compare with color developed by 1 ml. of *o*-tolidine reagent in 5 minutes.

1	9	8
2	16	8
3	22	8
4	28	8
5	33	8
6	38	8
7	44	8
8	50	8
9	57	8
10	66	8

Compare with the color developed by 5 ml. of *o*-tolidine reagent in 15 minutes.

water, and 10 times the quantity listed should then be used. Prepare the series of artificial standards with these solutions according to Table 11.

Chlorine by Benzidine

Free chlorine in the absence of permanganate ion can be estimated from the bright green color obtained by reaction with benzidine hydrochloride. The color is much more intense and favorable for colorimetric comparison than that by the reaction of chlorine with *o*-tolidine. The color fades in about 2 minutes to an unstable greenish yellow. Comparison is made with artificial standards. Large amounts of sulfate interfere because of formation of insoluble benzidine sulfate.

Procedure. Measure a portion of sample to contain 0.001-0.01 mg. of free chlorine. Dilute to 100 ml. and add one drop of benzidine reagent. This is a solution of 2.3 grams of benzidine per 100 ml. of 1:20 hydrochloric acid. Mix and compare at once with the artificial standard by balancing.

Prepare the artificial standard empirically to match a solution containing 0.003 mg. of free chlorine per 100 ml. Mix a 15 per cent solution of copper sulfate and a 0.5 per cent solution of picric acid until the desired tint of blue-green is obtained. Dilute this until it matches the color developed from a freshly prepared 0.003 mg. free-chlorine sample.

Chlorine by Dimethyl-*p*-phenylenediamine

Free iodine, bromine, or chlorine gives a red color with dimethyl-*p*-phenylenediamine. Since iodine and chlorine solutions of the same normality have the same color intensity, a chlorine solution of unknown strength may be compared with a standard iodine solution.

Procedure. To 50 ml. of solution to be examined add 1 ml. of 50 per cent acetic acid and 2 ml. of 16 per cent sodium acetate solution. Similarly treat a standard at the same time. Then add 10 drops of a 0.1 per cent alcoholic solution of dimethyl-*p*-phenylenediamine to each and dilute to 100 ml. The color develops in 10-15 minutes and must not stand more than 30 minutes before comparison. As standard prepare a standard iodine solution containing 0.1269 gram per liter. This is equivalent to 0.03546 mg. of chlorine per ml.

Chloramine by *o*-Tolidine

The usual *o*-tolidine method for free chlorine can be used to estimate chloramine. Nitrite ions interfere.

Chloramine by Nessler's Reagent

Chloramine exists in solution at a pH above 4.4. Above pH 8.5 only the monochloramine is present, between 4.4 and 8.5 both mono- and dichloramine are present. The chloramine content is measured by acidifying one sample and rendering another alkaline. In the acid solution chloramine is converted to give two-thirds of the nitrogen as ammonia and the balance as nitrogen trichloride. The ammonia formed is then estimated by Nessler's reagent.[2] The ammonia in the alkaline solution serves as a correction for the ammonia originally present in the solution. The color is 90 per cent developed within 1 minute, after which destruction in the alkaline tube alters the results. The method is not as sensitive as the *o*-tolidine method. Probably even greater accuracy would be obtainable by reading the alkaline tube within 1 minute and the acid tube in 5-10 minutes.

Procedure. Transfer 50 ml. of sample to each of two 50-ml. Nessler tubes. Add 0.2 ml. of 1:10 sulfuric acid to one to lower the pH below 4.4. Mix and let stand for 1 minute. If the pH of the original solution is below 8.6, add 0.1 ml. of 5 per cent sodium carbonate solution to raise the pH. To each add 2 ml. of Nessler's reagent (page 814). Invert and compare with standards (page 816) within 1 minute. The difference in ammonia by the 2 readings gives chlorine as chloramine by the following formula:

$$\text{Ammonia} \times 3/2 \times 35.4/14 = \text{chlorine as chloramine.}$$

The value of the factor is 3.8.

Miscellaneous

The reaction of chlorine in oxidizing 3,3′-dimethyl-4,4′-diamino biphenyldihydrochloride at pH 4 to a colored compound is particularly applicable for small amounts.[3] Two mols of free chlorine react with a mol of methyl orange to give a method sensitive to 0.1 ppm. of chlorine.[4] Near pH 3 the color of the methyl orange solution is a function of chlorine content rather than pH. Chloramine does not interfere but no

[2] Paul C. McNamee, *Ind. Eng. Chem., Anal. Ed.* **7**, 233-4 (1935).
[3] Harry Scharer, U. S. Patent 2,385,471 (1945).
[4] Michael Tarus, *Anal. Chem.* **19**, 342-3 (1947).

more than 0.05 ppm. of manganic ion is permissible since the reaction is equally sensitive to it and to free chlorine.

To a tube containing 100 ml. of sample add 0.1 ml. of 1:1 hydrochloric acid and 3 ml. of 0.005 per cent methyl orange. Compare against a series of methyl orange standards reading in ppm. of chlorine as equaling 0.217 (ml. of 0.005 per cent methyl orange solution) plus 0.04. Results are sensitive to 0.1 ppm. of chlorine.

CHAPTER 50

CHLORIDE

Chlorides are widely distributed, if only because of the wide dissemination of common salt. Therefore the determination is of importance in biological fluids such as blood and urine, and in water where a high content may indicate contamination by either sea water or sewage. In a different field, the highly accurate estimation of chloride is used for atomic weight determination. The usual method is nephelometrically as silver chloride, although other methods are included, notably indirect methods depending on the estimation of the amount of silver combined as silver chloride. The nephelometric estimation of silver chloride is well standardized so that little or no work has been done on it in years.

Samples

Blood. Transfer 5 ml. of the sample to a 100-ml. volumetric flask. Dilute with 35 ml. of water and mix. Add 5 ml. of a 10 per cent solution of commercial sodium tungstate and mix. Add 3.5 ml. of 1:35 sulfuric acid, close with a rubber stopper and shake vigorously a few times. Only a few air bubbles should form as a result of shaking.

Not over 10 mg. of potassium oxalate should be present in the sample. Citrate must be absent. If coagulation has proceeded properly the precipitate changes slowly from pink to dark brown. If the dark brown color does not appear, too much oxalate is present or citrate was used as an anticoagulant in the sample. In that case add 1:35 sulfuric acid drop by drop, shaking vigorously after each addition, until coagulation is complete. Wet a filter large enough to contain the entire contents of the flask, with a few ml. of the solution. Pour the contents into the filter and cover to prevent evaporation. If the filtrate is not perfectly clear return the first few ml. to the funnel. The filtrate so obtained should be neutral or just faintly acid to Congo red paper. It can be preserved for several days by addition of 1-2 drops of toluene.

Urine. Transfer 1 ml. of urine to a centrifuge tube. Add about 5 ml. of water, 1 ml. of 1:3 nitric acid, and 2 ml. of 10 per cent silver nitrate solution. Mix well and centrifuge. Decant and make sure that the

supernatant liquid shows no test for chloride. Wash the precipitate 3 times with 5 ml. of distilled water and use as sample for the determination of chlorides indirectly, preferably by estimating the silver in silver chloride.

Standard

Dissolve 0.1649 gram of sodium chloride in water and dilute to 100 ml. This contains 1.0 mg. of chloride per ml. Dilute 10 ml. of this solution to 1 liter. It then contains 0.01 mg. per ml.

Chlorides Nephelometrically as Silver Chloride

The classical method for the determination of traces of chlorides is nephelometrically as silver chloride.[1] The method is widely applicable for accuracy varying from rough estimation to accurate determination. A suitable concentration is 0.2 mg. per 100 ml. of sample after dilution. Other ions forming insoluble silver salts, such as bromides and iodides, must be absent. Errors of the method have also been discussed in detail under silver.

Procedure.[2] *Balancing.* Dilute or concentrate a sample containing about 0.2 mg. of chloride to 20 ml. Take a corresponding standard. Add 10 ml. of 1:100 nitric acid to each, mix, and add 10 ml. of a 0.1 per cent silver nitrate solution. Mix and place in a water bath at 40° for 30 minutes or more. Cool rapidly to room temperature and compare within 30 minutes in a nephelometer.

If maintained at 20° the opalescence increases slowly to an almost constant value in an hour. Heating to 60° not only gives full development in less time but results in a greater opalescence, which falls off on continued heating. After 30 minutes coagulation begins. Heated at 40° this coagulation does not occur and constant opalescence is obtained after 30 minutes. Further heating at this temperature has no effect. The solution must be cooled before reading. Exposure to diffused daylight has a perceptible effect, especially on the more concentrated solutions. If greater sensitivity is desired dilute to 100 ml. with chloride-free ethanol, the final solution containing about 50 per cent of alcohol.

Duplication. This is considerably less accurate than the balancing

[1] T. W. Richards, *Proc. Am. Acad. Arts Sci.* 30, 385 (1894); T. W. Richards and R. C. Wells, *Am. Chem. J.* 31, 235-43 (1904); *J. Am. Chem. Soc.* 27, 483 (1905); R. C. Wells, *Am. Chem. J.* 35, 99-114 (1906); T. W. Richards, *ibid.* 35, 510-13 (1906).

[2] A. B. Lamb, P. W. Carleton and W. B. Meldrum, *J. Am. Chem. Soc.* 42, 253 (1920).

method. Transfer the sample to a 50-ml. cylinder and an equal volume of water containing the same known impurities to another cylinder. Dilute each to 20-30 ml. and add 5 ml. of concentrated nitric acid and 2 ml. of 0.1 per cent gelatin solution. Dilute the sample to 49 ml. and add 1 ml. of 1.7 per cent silver nitrate solution and mix. Dilute the standard to about 45 ml. and add 1 ml. of the 1.7 per cent silver nitrate solution. Add the standard sodium chloride solution to this standard until the opalescence of the standard matches that of the sample, comparing them against a black background. Adjust the volume as usual.

Chlorides Indirectly by Silver Chromate

An indirect method for determining chlorides depends on the reaction of solid silver chromate with chloride to liberate sodium chromate. This may be estimated either by duplication or balancing. The chromate may also be determined by conventional methods such as the amount of iodine which it liberates in acid solution or by diphenylsemicarbazide. As a micro method, the latter is stated to be accurate to 3 per cent on 0.0004 mg. of sodium chloride.

Procedure. To prepare the reagent, add 200 ml. of 5.5 per cent potassium chromate solution slowly to 100 ml. of boiling 10 per cent silver nitrate solution. The precipitate will settle rapidly. Continue to add chromate solution drop by drop until it is in slight excess as shown by a yellow color in the supernatant liquid. Filter on a Büchner funnel, wash with distilled water, and air-dry. Unless the solution is boiling while silver chromate is precipitated, the bichromate will form.

Pipet a 10-ml. aliquot of sample into a centrifuge tube. Add about 0.1 gram of magnesium carbonate to neutralize excess acidity, more if necessary. Stir and add about 0.05 gram of dry reagent. If all the red particles of silver chromate added to the sample disappear, add more. Centrifuge for 2 minutes. Decant carefully through a small filter paper into a 25-ml. volumetric flask. Add 10 ml. of water to the residue in the tube and centrifuge for 5 minutes. Filter into the flask. If the filtrate is slightly turbid with silver chloride, add 1 ml. of 1:20 ammonium hydroxide. Dilute to 25 ml. The centrifuging operation may be eliminated and time saved by direct filtration of the suspension of silver chromate after the color has been developed.

Estimation by Duplication. Transfer the solution of developed sample to a comparison tube. Into a second 25-ml. comparison tube put 5 ml. of 0.1 per cent sodium chloride solution and dilute nearly to volume. Add a standard solution containing 0.2738 gram of potassium chromate

per liter, until the color matches that of the sample. Each ml. of this solution is equivalent to 0.1 mg. of chloride. Adjust the volume of standard to match that of the sample.

Estimation by Balancing. Prepare a 25-ml. standard containing 5 ml. of 0.1 per cent sodium chloride solution together with a known amount of the above potassium chromate solution so that the color nearly matches that of the sample. Dilute exactly to volume and compare by balancing. As yellow colors are rather hard to match, a blue glass is advantageously interposed.

Estimation by Iodide. Transfer the filtrate to a 25-ml. volumetric flask. The volume of filtrate and washings must not exceed 22 ml. Add 1 ml. of 50 per cent potassium iodide solution and 1 ml. of 1:10 sulfuric acid. Dilute to 25 ml. with distilled water and mix well. Compare the results with those of a standard containing 0.8350 gram of potassium chromate per liter. The valuo is approximately that of a sodium chloride solution coataining 0.5 mg. per ml.

Miscellaneous

As an indirect method an excess of silver nitrate may he added, the resulting silver chloride coagulated and separated. This precipitate is then dissolved and the silver in it estimated colorimetrically by any conventional method for silver. As a variant, the amount of silver nitrate is measured and the excess silver determined after separation of silver chloride. To a 10-ml. sample made slightly acid with nitric acid add 0.1 per cent silver nitrate solution until the opalescence no longer increases. Compare the turbidity with an artificial standard turbidity (Vol. 1, page 61). Estimate the chloride content from data obtained by comparing standard chloride solntions with the turbidity standards.

A solution containing chloride ion with ferrous sulfate when applied to a silver ferrocyanide suspension gives a color determinable by comparison with a series of standards.[3] Very small amonnts of chloride are estimated by the effect on snppression of the reaction of mercuric ion with diphenylcarbazide.[4] The color developed is stable for 5-30 minutes and conforms to Beer's law. If ammonia exceeds 12 mg. per liter, it should be neutralized. The optimum pH is 4.0. Up to 0.5 mg. of iron does not interfere. Under optimum conditions, 0.025 mg. of chloride can he detected.

[3] Clément Duval and Gabriel Mazars, ***Compt. rend.*** 208, 579-80 (1939).

[4] Yu. Yu. Lur'e and Z. V. Nikolaeva, ***Zavodskaya Lab.*** 12, 161-70 (1946).

CHAPTER 51

CHLORATES AND PERCHLORATES

Chlorates and perchlorates are among the most widely used strong oxidizing agents in acid solutions. The methods, in general, measure oxidizing properties. Alternatives are to reduce to chlorides and measure by the methods in the preceding chapter, provided chlorides as such are not originally present. In organization, this chapter is somewhat different from the majority, maintaining the subdivision into separate unrelated methods of the second edition. Thus it is assumed that a sample free from interfering substances is available.

Chlorates by Aniline Hydrochloride

Oxidation of aniline hydrochloride is a general reaction for estimation of small amounts of many oxidizing agents. A violet color develops at once, which changes to blue. The method is applicable to chlorates in the absence of other oxidizing agents. The method will detect 0.007 mg. of potassium chlorate. The same color is produced by any oxidizing agent capable of oxidizing aniline hydrochloride under the conditions of the method. Interfering oxidizing agents include chlorine, hypochlorites, chlorates, hypobromites, bromates, iodates, hydrogen peroxide, peroxides of sodium, barium, manganese and lead, chromates and bichromates, manganates and permanganates, vanadates, ferricyanides, and per salts in general. Substances more easily oxidized than aniline interfere. These include unstable chlorides, nitrites, citrates, tartrates, saccharates, a large number of organic reducing compounds, ferrous compounds, arsenites, sulfites, etc.

Procedure. Neutralize 5 ml. of the sample solution with acetic acid or with sodium carbonate. Place in a comparison tube with a suitable amount of 0.5 per cent potassium chlorate solution in another tube, diluted to 5 ml. Prepare a concentration of reagents according to the strength of the solution being examined. For quantities of chlorate between 0.1 and 2 mg. in 5 ml., dissolve 5 grams of pure aniline hydrochloride in 100 ml. of 1:2 hydrochloric acid. For quantities of chlorate

between 0.5 and 7 mg. in 5 ml., dissolve 5 grams of pure aniline hydrochloride in 100 ml. of 1:3 hydrochloric acid.

To sample and standard add 20 ml. of reagent solution, according to the strength anticipated. Compare after 25 minutes if 0.1-2 mg. of chlorate are present or, after 15 minutes, if 0.5-7 mg. of chlorate are present. They may be compared by dilution with an accuracy to 1 per cent.

Chlorates by Ammonium Thiocyanate

The reaction of chlorate ion to oxidize ammonium thiocyanate to a lemon to cadmium yellow may be utilized on test papers for estimation of chlorate.[1] Only a drop of sample is needed and it need not be clarified. As would be expected the method is only roughly quantitative. It will under optimum conditions detect 0.01 mg. of chlorate per ml. Large amounts of nitrate, phosphate, sulfate, cyanide, thiosulfate, and sulfite limit the sensitivity to about 0.1 mg. per ml. The color is due to canarine and pseudothiocyanic acid with small amounts of hydropseudothiocyanic acid and isoperthiocyanic acid. A similar color is produced by bromates, iodates, peroxides, persulfates, hypohalites and halogens. Perborates and borax do not interfere. Permanganates and dichromates mask the color and iron, cobalt, copper and molybdenum produce characteristic colors. The solution may be 0.1 N with sulfuric, nitric, acetic or oxalic acids.

Procedure. Impregnate filter paper with 25 per cent ammonium thiocyanate solution. Dry with maximum air circulation out of contact with metal, at not over 70°. Protect from light and dust. A slight pink color due to a trace of iron will disappear on heating for use.

Dry the paper at 60° for 10 minutes and cut the part showing no discoloration into strips. To these strips add a drop of the sample solution and of suitable standards. Heat on a clean glass surface at 95-105° until no further intensification of color occurs. This is usually 5-30 minutes. Compare the sample with the standards for estimation of the chlorate content.

Perchlorates by Methylene Blue

Methylene blue gives a violet precipitate with perchlorates which is soluble in hot water, although the method is not fully satisfactory. After

[1] H. R. Offord, *Ind. Eng. Chem., Anal. Ed.* **7**, 93-5 (1935).

treatment with a standard excess of methylene blue solution, the paler the color the greater the amount of perchlorate originally present. This inverse relation is used for colorimetric estimation by comparison with a series of standards. Persulfates give a rose precipitate. By allowing time for the precipitate to settle the color of the supernatant liquid can be compared, or by addition of a large excess of zinc sulfate solution precipitation can be prevented and the comparison made after 15 minutes.[2] The reaction is applicable to other dyes containing the quaternary ammonium group such as crystal violet and malachite green.

Iodides interfere but can be removed. Fluorides, chlorides, hypochlorites, chlorates, bromides, bromates, iodates and periodates, sulfates, nitrites, nitrates, phosphates, borates, perborates, carbonates, and percarbonates do not interfere. Acid chromates interfere but may be precipitated by lead acetate in neutral solution. A precipitate is also given by dilute acid solutions, permanganates, ferricyanides, metavanadates, molybdates, and tungstates.

More than 5 per cent of sodium nitrate or 10 per cent of potassium nitrate interferes by precipitating some of the methylene blue. The method is intended to be used for relatively large amounts of perchlorate, such as are found in Chili saltpeter, 0.1 to 7 per cent. Chlorates containing perchlorate require a special procedure.

Procedure. *General.* For less than 0.2 per cent of perchlorate take 10 ml. of the prepared sample solution, for 0.2 to 0.5 per cent take 5 ml., and for over 0.5 per cent take 1 ml. Transfer this to a test tube and dilute to 20 ml. Put portions of 1-5 ml. of a 0.1 per cent solution of potassium perchlorate into the same-size test tubes as used for the sample. To these standards add the same volume of 20 per cent perchlorate-free solution of the same kind of salt as is being examined. For examination of Chili saltpeter this would be pure sodium nitrate. Dilute each to 20 ml.

To sample and standards add 1 ml. of a 0.3 per cent aqueous solution of methylene blue. Let sample and standards stand for several hours in a cool place for precipitation. Compare the color of the sample with the series of standards prepared at the same time.

Perchlorate over 0.3 Per Cent. Mix 5 ml. of a 0.032 per cent solution of methylene blue with 20 ml. of a 50 per cent solution of crystallized zinc sulfate. Add 0.2 ml. of the prepared sample solution, mix, and

[2] F. L. Kahn, *Z. angew. Chem.* 39, 451-4 (1926).

compare after 15 minutes with standards similarly treated. The colors obtained will correspond to the following series:

0%	Dark blue
0.1%	Slightly lighter
0.1-0.3%	Decreasing color intensity and increasing turbidity
0.4-0.7%	Reddish violet with gradually decreasing turbidity
0.7-1.0%	Further small changes.

The sensitivity is improved by comparison through a yellow glass filter. These standards keep well if properly protected and shaken before use.

Perchlorate 0.05 to 0.3 Per Cent. Mix 0.1 ml. of 1.6 per cent methylene blue solution with 25 ml. of 50 per cent crystallized zinc sulfate solution. Add 0.2 ml. of the prepared sample solution and compare after 15 minutes with a series of standards similarly prepared.

Perchlorate under 0.05 Per Cent. Mix 0.1 ml. of 1.6 per cent methylene blue solution with 25 ml. of saturated zinc sulfate solution. Add 0.2 ml. of the prepared sample solution. Compare with a series of standards similarly prepared. Amounts of the order of 0.02 to 0.04 per cent are discernible by this procedure.

Chlorate Containing Perchlorate. Prepare a reagent by mixing 0.1 ml. of 1.6 per cent methylene blue solution with 25 ml. of 50 per cent crystallized zinc sulfate solution. To 0.2 ml. of prepared sample solution, or 0.1 ml. of sample solution and 0.1 ml. of water, add 0.1 ml. of 40 per cent potassium nitrate solution. Add 5 ml. of reagent and mix. Compare with standards.

By using a more concentrated zinc sulfate solution as small an amount as 0.1 per cent of perchlorate in potassium chlorate can be detected.

As standard dissolve 0.1393 gram of potassium perchlorate in water and dilute to 1 liter. Dilute 100 ml. of this solution to 1 liter. Each ml. of the first solution contains 0.1 mg. of perchlorate radical and each ml. of the final solution 0.01 mg.

Perchlorates by Nitrosodimethylaniline

The action of perchlorates on nitrosodimethylaniline is adapted to estimation of perchlorates, particularly in sodium nitrate. Iodides

must be removed with silver oxide. Iodates and periodates do not interfere.

Procedure. Dissolve a 1-gram sample in 20 ml. of water. If iodides are present, add sufficient freshly precipitated silver oxide to remove them and filter. Transfer to a Nessler tube. Add 2 ml. of a 0.1 per cent solution of nitrosodimethylaniline in water, mix, and let stand. Prepare a series of standard tubes at the same time using 1-10 ml. of the standard perchlorate. Treat in the same way and compare after allowing several hours for full development of color.

CHAPTER 52

BROMIDE

Bromide as compared with chloride is rare. When it is present in the absence of chloride, the nephelometric method for chloride is applicable. Some mineralogical occurrences are important, and biologically the occurrence is related to the soporific activity of triple bromides. Generally the methods are related to the displacement of bromine by the stronger halogen, chlorine.

Samples

Inorganic. Dissolve a suitable amount of sample so that 1 ml. contains about 0.2 mg. of bromide. Alkaline fusion methods are permissible, provided no oxidizing agent is added for getting the sample into solution.

Organic. Ash without addition of oxidizing agents. Dissolve the ash and dilute to a volume in which the bromide concentration will be about 0.2 mg. per ml.

Brine. Make 100 ml. of sample or other suitable volume alkaline with sodium carbonate and evaporate to dryness. Take up with 25 ml. of distilled water and filter into a 250-ml. volumetric flask, washing thoroughly. Add 5 ml. of 1:1 sulfuric acid and test for acidity. If not acid, continue the addition until acid to Congo red paper. Dilute to volume for use of aliquots in the determination of bromine by extraction.

Blood. Mix 2 ml. of blood and 8 ml. of 10 per cent trichloroacetic acid solution in a tube and centrifuge when well coagulated. Filter and use 5 ml. of filtrate as sample for determination of bromide by gold chloride.

Removal of Reducing or Oxidizing Agents. Render the sample alkaline, evaporate to dryness and, if necessary, ignite. Take up with a suitable volume of water and proceed.

Removal of Iodides. *To 10-15* ml. of sample add 2 ml. of 1:35 sulfuric acid and 1 ml. of 3.5 per cent sodium nitrite solution for each

60 mg. of iodide present. Boil gently with stirring until the solution becomes colorless. Wash the sides of the flask with water to replace that lost by evaporation and add a few more drops of sulfuric acid and nitrite solution. If a color appears, add more acid and nitrite and repeat the boiling. When the solution finally remains colorless, boil 2 minutes longer, wash the sides of the flask, and cool.

Standard

Dissolve 1.4891 grams of potassium bromide or 1.2826 grams of sodium bromide in water and dilute to 1 liter. Each ml. will contain 1 mg. of bromine.

Bromide by Chlorine Water

Bromide may be determined by the red color of free bromine liberated by a very small amount of chlorine water. Iodide should not be present in a concentration over 50 per cent of that of the bromide and preferably not over 30 per cent. When iodide is low, accuracy to 1 per cent is attainable. If the sensitivity must be increased, the bromine may be extracted with carbon tetrachloride.

Procedure. *Direct Reading.* Take suitable volumes of sample and standard solutions in 100-ml. Nessler tubes. These will desirably contain about 5 mg. of bromide. Dilute sample and standard to about 75 ml. Add 10 ml. of 1:1 sulfuric acid and mix. Add a saturated solution of chlorine dropwise to each until a maximum color is obtained. This will normally require about 5.0 ml. If the brown color of iodine appears, add more chlorine water until the interfering color is removed. Dilute each to 100 ml. Balancing methods are permissible if the standard and sample nearly match. The standard must be fresh, as it fades on standing.

Extraction. If the color developed is not sufficiently intense, extract the bromine from sample and standards by shaking with 10 ml. of carbon tetrachloride. Separation of the solution from carbon tetrachloride is most conveniently carried out by filtration on a wet paper, later puncturing the paper to get the carbon tetrachloride solution of bromine. Better results will be obtained if filtration is carried out in a darkened room.

Compare by balancing or dilution with carbon tetrachloride to get an approximate bromine estimation Repeat the determination with

another aliquot of the sample solution using a standard or set of standards based on the first estimation.

Bromide by Fuchsin

Bromine, liberated from bromide by chlorine water, forms a violet color in a sulfuric acid solution of basic fuchsin. The intensity of color is proportional to the amount of bromine present.[1] For estimation extract the color with isoamyl alcohol. The method will detect 0.007 mg. of bromine. The sample and standard should not differ by more than 50 per cent.

Procedure. To prepare the reagent, transfer 100 ml. of 5 per cent sulfuric acid to a 250-ml. cylinder, add 10 ml. of a 1 per cent fuchsin solution, and shake. Let stand for 2 hours at which time the fuchsin should be entirely colorless. Transfer 20 ml. of the sample and standard solutions to separatory funnels. Add 2 ml. of fresh 5 per cent chlorine water and 20 ml. of the fuchsin reagent. Mix vigorously and add 5 ml. of isoamyl alcohol. Shake and separate the isoamyl alcohol layers for comparison.

Bromide by Schiff's Reagent

Schiff's reagent, the color of which has been developed with a hypochlorite solution, gives a color with bromine suitable for colorimetric estimation by the series-of-standards method with accuracy to 5 per cent.

Procedure. To 100 ml. of a 1 per cent fuchsin solution, add 80-90 mg. of sulfur dioxide dissolved in water and dilute to 1 liter. It will become colorless or faintly yellow in 24-36 hours and is stable for several days if kept cool and in the dark. Prepare an oxidizing solution by dissolving 1 gram of calcium hypochlorite containing about 0.4 gram of available chlorine in water and dilute to 500 ml. Mix 0.1 ml. of 1.49 per cent potassium bromide solution and 1 ml. of 1:4 sulfuric acid. To this mixture in 3 separate tubes, add 0.2, 0.25 and 0.3 ml. of the oxidizing solution. Add 1 ml. of Schiff's reagent to each of these. The oxidizing solution should be of such a concentration that one of them will have a *gray-violet* color. That is the standard volume of oxidizing solution to add. If necessary, dilute the oxidizing solution to fall within that range.

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[1] Roberto Indovina, *Biochem. Z.* **275**, 286-92 (1935); *Boll. soc. ital. biol. sper.* **10**, 189-91 (1935); cf. R. Casares Lopez, *Farm. moderna* **46**, 55-7 (1935); *Ann. fals.* **28**, 115-16 (1935).

Measure 0.1, 0.2, 0.5, 0.8 and 1.0 ml. of the unknown into separate test tubes. To each add 1 ml. of 1:4 sulfuric acid, the standard volume of oxidizing agent and 1 ml. of Schiff's reogent. One should be groy-violet ond contains obout 0.1 mg. of bromide in the volume taken. Check this by taking the same amount of the sample in three tubes. Treat in the same woy except that one receives 0.05 ml. less of oxidizing agent and should be yellow. One should duplicate the previous gray-violet. A third should have 0.05 ml. more of oxidizing agent and should be distinctly violet. .

Prepare a series of standards containing 0.075, 0.1 and 0.125 mg. of bromide. Dilute each to the volume of the sample solution. To each add the same reagents as to the sample which gave a gray-violet color and compare with that sample.

Bromide by Fluorescein

The reaction of bromine with fluorescein to form eosine may be used for quantitative estimation of bromides, even in the presence of large amounts of chlorides. The greenish yellow color is altered to pink. Fluorescein paper is also used for the estimation, evolving the gas in the general style of the Gutzeit method. The bromine is best liberated by a suitable oxidizing agent which will liberate bromine but not chlorine. More drastic oxidation can be used and results still estimated. Iodine is first distilled from acid solution containing ferric alum, then the bromine liberated by potassium permonganate.

Procedure. Prepare a 3.3 per cent solution of fluorescein in 0.4 per cent sodium hydroxide solution. Dilute 5 ml. of this to 1 liter as reagent. To prepare a buffer solution, mix 1 volume of an 8 per cent solution of sodium acetate with 0.1 volume of 1:16 acetic acid. This buffer should fall in the range of pH 5.5-5.6. Add 1 drop of the fluorescein reagent to 1 ml. of sample solution. Add 3 drops of buffer solution and 1 drop of 0.1 *N* sodium *p*-tolylchlorsulfonaminate. Mix and let stand for 1 minute. Add 1 drop of 5 per cent sodium hydroxide solution containing 0.5 per cent of sodium hyposulfite. This latter addition stops the development of color. Compare with a series of standards.

Bromide by Phenol Red

Phenol red, like fluorescein, absorbs small quantities of bromine to form an indicator of the bromophenol blue type. This takes place at pH 8.7-8.8 in u borax buffer solution in the presence of calcium hypochlorito

and is suitable for colorimetric estimation.[2] Any chloro-compound formed does not differ in color from the phenol red at pH 5.0-5.4, at which the comparison is made. The color is about constant for 0.018-0.03 mg. per ml. and above that concentration the indicator is attacked. Reducing agents including ammonia interfere. Iodides interfere because they behave like bromide.

Procedure. To prepare a reagent, extract calcium hypochlorite, $Ca(OCl)_2$, with water and filter. Dilute the filtrate to an oxidizing value of 0.1 *N*, within 10 per cent, by titration. For use dilute to one-tenth strength, 0.01 *N*. For the acetate buffer, dissolve 30 ml. of glacial acetic acid and 68 grams of sodium acetate trihydrate in water and dilute to 1 liter. This has a pH of 4.6-4.7. It need not be free from bromide.

Not over 0.004 Mg. of Bromide per Ml. Pipet 1 ml. of the sample solution into a 5-ml. vial. Add 0.05 ml. of a solution containing 10 mg. of phenol red and 1 ml. of 0.4 per cent sodium hydroxide solution per 100 ml. Add 0.2 ml. of saturated borax solution. Add 0.2 ml. of 0.01 *N* calcium hypochlorite reagent, mix and let stand for exactly 4 minutes with occasional shaking. Add 0.05 ml. of 0.1 *N* sodium arsenite solution and 0.2 ml. of acetate buffer. Compare with standards prepared in the same way. The color is yellow below 0.001 mg., reddish from 0.0015-0.002 mg. and blue-violet above 0.0025 mg. The accuracy in this range is 15 to 20 per cent.

From 0.003 to 0.01 Mg. of Bromide per Ml. Follow the preceding procedure using a 10-ml. sample in a 20-25 ml. vial, 0.2 ml. of phenol red, 2.0 ml. of borax solution, 0.2 ml. of 0.1 *N* hypochlorite solution, 0.5 ml. of arsenite solution, and 1.5 ml. of acetate buffer. Accuracy of about ±10 per cent is obtainable.

Bromide by Gold Chloride

A solution of gold chloride gives an orange to red color with a solution of bromide, the color varying with the concentration of bromide. The method was worked out for use with blood samples to guard against bromide intoxication. The error is less than 5 per cent.

Procedure. Mix 5 ml. of sample and 1 ml. of a 0.5 per cent solution of gold chloride. A lemon yellow color is a negative reaction. Read the transmittance against a reagent blank at 440 mμ and compare with a calibration curve.

[2] V. A. Stenger and I. M. Kolthoff, *J. Am. Chem. Soc.* 57, 831-3 (1935).

CHAPTER 53

IODINE AND IODINE COMPOUNDS

MINUTE amounts of iodine are important. So defined, samples range from minerals to foods and biologicals. Fortunately, very minute amounts of iodine are determinable. One method of magnifying the effect is to oxidize the iodine of the sample to iodate and use it to oxidize hydriodic acid, thus liberating 6 times the amount of iodine which was in the sample. The indefinite reaction with starch to form a blue color is usable in extreme cases, but isolation by solvent extraction and reading the color so obtained is preferable. With larger amounts of iodine the color is read in aqueous solution.

SAMPLES

Soil and Rock.[1] Iodine present in soil or rock is volatilized on heating. A furnace capable of heating a 100-gram sample in a closed tube is required. Place 25-100 grams of soil or small pieces of rock in a boat in the furnace. Connect the exit of the combustion tube with 3 gas bottles; loosely stopper the inlet end with an alundum crucible. Fill the wash bottles with a 5 per cent aqueous solution of potassium carbonate. Connect the last wash bottle to a suction pump.

Bring the furnace up to 1100° in about 2 hours and continue to heat for 2 hours, drawing air through at a slow rate. Rinse the contents of the wash bottles into a porcelain dish and evaporate to dryness. Take up the residue with 10 ml. of water and filter into a small porcelain dish. Evaporate to dryness and ignite gently to destroy organic matter. When cool, dissolve in 0.2 ml. of water and filter into a small separatory funnel. Wash the filter with 0.1 ml. of water, then with absolute ethanol. Add absolute ethanol until two layers are formed. Shake and separate the ethanol layer. Repeat twice. Evaporate the alcoholic filtrates to dryness and take up with 1 ml. of water. Acidify with a drop of 1:1 sulfuric acid and dilute to 10 ml. for use as sample for the determination of iodine by solvent extraction.

[1] J. S. McHargue, D. W. Young and W. R. Roy, *Ind. Eng. Chem., Anal. Ed.* 4, 214-16 (1932).

Water. Evaporate 3-6 liters of water containing 0.25 ml. of 0.01 per cent phenolphthalein solution and about 10 ml. of 0.6 per cent potassium carbonate solution, to about 150 ml. Keep the solution alkaline by means of added potassium carbonate solution if necessary. Filter to remove calcium carbonate and iron, and evaporate the filtrate nearly to dryness in platinum. Extract the residue of moist salts with 3 portions of 95 per cent ethanol. Evaporate the residue to dryness and ignite carefully. Moisten with 0.25 ml. of 0.6 per cent potassium carbonate solution and again extract with ethanol. Evaporate the combined alcoholic extracts to dryness. Ignite very carefully and take up in 1 ml. of water. Add 1 drop of 1:1 sulfuric acid for determination of iodine by solvent extraction.

Alternatively,[2] if the iodine content of water is very low, as much as 100 liters may be necessary as sample. The solution must remain alkaline to phenol red during the evaporation to prevent loss of iodine. For this large-scale evaporation prepare a clean barrel with a 1-inch pipe as exit tube to feed the pan used for evaporation. Add sodium carbonate so that the sample is alkaline. Evaporate rapidly in a large dish pan containing 2 grams of sodium bicarbonate, adding more sample water from time to time, to a volume of 1 liter. Filter and evaporate the filtrate to dryness. Powder and place in a silica, nickel, or iron boat in a silica tube. Heat to a dull red and lead the exit gases through 10 ml. of 10 per cent sodium hydroxide solution. Pass oxygen through the tube during the heating, which should be as brief as possible with complete combustion of the organic matter.

Evaporate the absorption solution and rinsings of the combustion tube to dryness. Mix with the ash and powder. Add 15 ml. of water and grind in a mortar. Filter and take an aliquot of 7.5 ml. Neutralize with concentrated hydrochloric acid, add 1 drop in excess and dilute to 10 ml. for determination of iodine by solvent extraction.

Salt Brines. To a 50-ml. sample add 2 grams of ferric sulfate and render distinctly acid with sulfuric acid. To a receiver add 10 ml. of 1 per cent sodium hydroxide solution and 5 ml. of 3 per cent hydrogen peroxide. Steam-distill the sample until no further evidence is shown of iodine distilling over. Evaporate the contents of the receiver to dryness and take up with 5 ml. of distilled water. Neutralize with 1:1 sulfuric acid, dilute to 25 ml., and use as sample for determination of the iodine by *o*-tolidine.

[2] J. F. McClendon, *J. Biol. Chem.* 60, 288 99 (1924).

Blood. Mix 5 ml. of oxalated blood with 1 ml. of 50 per cent potassium hydroxide solution in a porcelain crucible. Concentrate to a thick paste on the water bath and heat cautiously at 400-500°. The material should char completely in 40 minutes to give a dry black powder. Transfer this quantitatively to a porcelain boat and burn in a combustion tube in a stream of oxygen. Proceed cautiously by slow admission of oxygen. The combustion should require only 5-6 minutes. No significant loss of iodine occurs.

Extract the residue with 5 ml. of 95 per cent ethanol in a micro extractor for about 20 minutes. The alcoholic extract should be clear and colorless. Transfer the extract quantitatively to a gold dish and rinse the apparatus 3 times with 1-ml. portions of water. Render the solution alkaline with 5 drops of 50 per cent potassium hydroxide solution. Evaporate to dryness on a water bath and ignite the residue over a free flame until it is white. If necessary, moisten with water and repeat the drying and ignition.

Moisten the residue to a paste with 2 ml. of absolute ethanol and a drop of water. If not pasty when worked with an agate pestle, excess alkalinity is not present, iodine has been lost, and the estimation must be repeated. Transfer the alcoholic extract to a platinum dish. Wash the residual salt with 4 additional 2-ml portions of absolute ethanol. Add 5 ml. of water and evaporate to dryness on a water bath. Dissolve the residue in 1 ml. of water and transfer to a flask. Rinse the dish with four 0.2-ml. portions of water and use as a sample for determination of iodine by extraction

Urine. Evaporate 2.5-4 liters of urine with 10 grams of sodium hydroxide to a thick syrup. Transfer to a small iron pan and add 20 grams more of sodium hydroxide. Heat slowly until fumes are evolved. Let cool and grind the clinker in a mill. Let stand overnight in a beaker with 200 ml. of 95 per cent ethanol. Decant through a filter and wash by decantation several times with ethanol. Transfer to the filter and wash several times with ethanol.

Evaporate the filtrate until the major portion of the ethanol has been driven off. Add 3 grams of sodium hydroxide and evaporate to dryness in a nickel dish Heat until volatile matter is driven off and the residue charred. Extract with 25 ml. of water and filter. Evaporate to 5 ml. and add 2 drops of 0.1 N arsenious acid. Add 1:3 sulfuric acid until distinctly acid and use as sample for determination of iodine by solvent extraction.

Alternatively, neutralize a sample with lime water and add about 5 ml. of extract of jack-bean meal to hydrolyze urea. Add 5 grams of

excess lime and evaporate to a small volume. Transfer to a nickel boat and add sulfur and cerium oxide or thorium oxide. Evaporate to dryness and ignite in the apparatus described below for foodstuffs starting at "Wash the exit gases . . ."

Foodstuffs.[3] *High Fat Content.* A sketch of suitable apparatus is shown in Figure 24. Prepare a steel tube with one end water-cooled. The other has a screw feed for advancing the contents of the tube. Make the sample into a dried stick to fit the tube. Advance this slowly into a silica tube in which it is burned in oxygen at such a rate that no soot or tarry

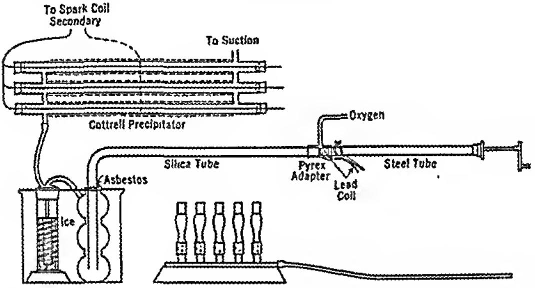

FIG. 24
Apparatus for Ashing Samples for Iodine Determination

matter is formed. An appropriate rate of feeding oxygen is 1.25 cubic feet per minute. Wash the exit gases io 4 wash bottles filled with 0.25 per cent sodium hydroxide solution and cooled with ice. Then pass the gas through a miniature Cottrell precipitator. As a slightly less efficient setup, use 4 additional wash hottles and a 25 × 500 mm. test tube packed with glass wool in place of the precipitator.

Grind the residual ash in a ball mill with an appropriate volume of 0.25 per cent sodium hydroxide solution and decant. Evaporate this extract, the contents of the absorption hottles, and the residue from the precipitator in a large heaker. When nearly dry, transfer to an evaporating dish and take to dryness. Ignite at a low temperature in a nickel hoat in a combustion tube and wash the exit gas with 0.01 *N* sodium hydroxide solution using a side-arm test tube. During this treatment, do not heat to a temperature at which the residue will fuse.

[3] J. F. McClendon and Roe E. Remington, *J. Am. Chem. Soc.* 51, 394-9 (1929).

Place the ash in a small beaker and add the absorption solution. Then add dropwise a mixture of 9 parts of 85 per cent orthophosphoric acid and 1 part of 0.1 N sulfurous acid until effervescence ceases. Boil gently for 5 minutes to expel sulfur dioxide. If the solution does not readily change bromophenol blue paper to yellow, add concentrated sulfuric acid drop by drop until it does. Dilute to 10 ml. with distilled water for determination of iodine by solvent extraction.

Low Fat Content. Prepare the sample in the form of compressed tablets. Burn these in an iron boat as an alternative to use of the injection device described. Follow the balance, starting with "Wash the exit gases . . ."

Fat.[4] Melt the fat and, if necessary, filter to free from water and suspended matter. Place in a large atomizer or lacquer spray. Spray into a heated silica tube having a 1-inch bore, 2 feet long with an elbow 1 foot long, using oxygen. The heating equipment is similar to that described for foodstuffs (page 730). It is essential that the tube be very hot and that a point just in front of the spray be kept particularly hot with a small blast flame or auxiliary heating coil. Place a platinum foil or spiral of heavy platinum wire in the tube as catalyst. Surround the end of the tube near the nozzle with a pad of moist asbestos cooled by dropping water. This also cools the nozzle and prevents carbonization on it.

As absorbents use 10 wash bottles in parallel, each containing 0.25 gram of sodium sulfite in water. This insures reduction to iodide. Follow these with a tower packed with glass wool. Draw the gases through the furnace and absorbers at 2 cubic feet per minute by use of a rotary air pump. In operation, if the ratio of oxygen to fat is too high, the fatty acids will distill over instead of burning, and, if too low, soot will form. As much as 500 grams of sample may be necessary. The amount used is determined by weighing the atomizer before and after the combustion. Soot and tarry products can be entirely avoided.

When combustion is complete, transfer the contents of the absorbers and the rinsings of the apparatus to a beaker. Evaporate to small volume, transfer to a nickel boat and evaporate to dryness. Ignite in a Pyrex tube. Use 5 ml. of water and a few mg. of sodium sulfite in a side-arm test tube to absorb volatilized iodine.

Place the boat in a very small test tube with the absorbing solution

[4] J. F. McClendon, Roe E. Remington, Harry von Kolnitz and Redding Rufe, *J. Am. Chem. Soc.* 52, 541-9 (1930).

excess lime and evaporate to a small volume. Transfer to a nickel boat and add sulfur and cerium oxide or thorium oxide. Evaporate to dryness and ignite in the apparatus described below for foodstuffs starting at "Wash the exit gases . . ."

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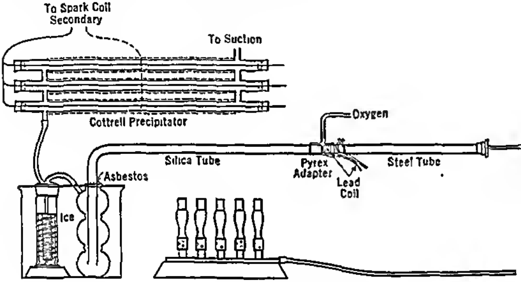

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[3] J. F. McClendon and Roe E. Remington, *J. Am. Chem. Soc.* 51, 394-9 (1929).

Place the ash in a small beaker and add the absorption solution. Then add dropwise a mixture of 9 parts of 85 per cent orthophosphoric acid and 1 part of 0.1 N sulfurous acid until effervescence ceases. Boil gently for 5 minutes to expel sulfur dioxide. If the solution does not readily change bromophenol blue paper to yellow, add concentrated sulfuric acid drop by drop until it does. Dilute to 10 ml. with distilled water for determination of iodine by solvent extraction.

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When combustion is complete, transfer the contents of the absorbers and the rinsings of the apparatus to a beaker. Evaporate to small volume, transfer to a nickel boat and evaporate to dryness. Ignite in a Pyrex tube. Use 5 ml of water and a few mg. of sodium sulfite in a side-arm test tube to absorb volatilized iodine.

Place the boat in a very small test tube with the absorbing solution

[4] J. F. McClendon, Roe E. Remington, Harry von Kolnitz and Redding Rufe, *J. Am. Chem. Soc.* 52, 541-9 (1930).

and rinsings of the tube for the ash to dissolve. Centrifuge from insoluble material and decant into a 30-ml. beaker. Add 5 drops of a saturated solution of sulfur dioxide to reduce iodate and 5 mg. of sodium azide to reduce nitrites. Neutralize with 85 per cent orthophosphoric acid, and add 4 drops in excess. If the odor of hydrazoic acid is not present, add more. Boil to drive off excess hydrazoic acid and sulfur dioxide. Transfer to a separatory funnel and dilute to 10 ml. for determination of iodine by solvent extraction.

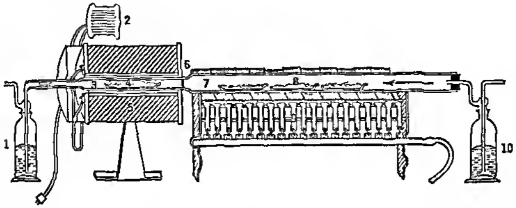

FIG. 25

Diagram of Apparatus for Combustion of Samples of Vegetables for Iodine Determination

1. Absorption bottle containing 5 per cent potassium carbonate solution. (2 bottles used.)
2. Rheostat.
3. Silica catalyst tube.
4. Platinized asbestos catalyst.
5. Electric tube furnace.
6. Asbestos cement seal.
7. Silica combustion tube.
8. Alundum boats containing samples.
9. Gas combustion furnace.
10. Wash bottle containing 10 per cent potassium hydroxide solution.

Apparatus for another method[5] of ashing is shown in Figure 25. Potassium carbonate solution is used for absorption; for this equipment sodium bisulfite is unsatisfactory.

Weigh 50 grams of air-dried sample into a porcelain dish and mix with 10 grams of finely pulverized calcium oxide and 10 grams of finely pulverized copper oxide. Transfer to alundum boats and place in a combustion furnace. Connect the absorption bottles on the left with a suction pump. Turn on the electric furnace and when the tube 3 attains a red heat, start the suction pump and light the first gas burner at the

[5] J. S. McHargue, D. W. Young and R. K. Calfee, *Ind. Eng. Chem., Anal. Ed.* 6, 318-9 (1934).

left of the series, 9. The heat from this should set the contents of the first boat on fire. The combustion is maintained at a regular rate by igniting other burners of the gas furnace. Any unburned vapors from the sample are drawn over the catalyst 4 and completely burned. The iodine vapors are absorbed in the gas wash bottles.

When combustion is complete turn off the heat and cool with a current of air drawn through the system. Turn off the suction and remove the boats. Digest the ash with hot distilled water. Filter and combine the filtrate with the potassium carbonate solution from the absorption flasks. Evaporate to dryness and take up with a minimum volume of water so that the solution is nearly saturated with potassium carbonate. Transfer to a separatory funnel and add sufficient 95 per cent ethanol to form 2 layers. Shake vigorously for about 10 minutes and separate the alcoholic layer. Repeat this extraction 3 times. Combine the alcoholic extracts containing the iodide and evaporate to dryness at a rate to avoid spattering. Dissolve the residue in a few drops of water and filter.

Add 3 ml. of a saturated solution of sulfurous acid to the sample in a separatory funnel and make it distinctly acid with 1:4 sulfuric acid. Stopper and shake for 1 minute to insure complete reduction to iodide. Transfer to a beaker and heat gently to drive off sulfur dioxide. Use this solution as sample, diluting if desirable, for the determination of iodine by solvent extraction.

Thyroid.[6] Thyroid free from fat may be mixed with potatoes of known iodine content and analyzed by the method given (page 730) for foodstuffs. A modified method designed solely for organic matter high in iodine is preferable.

Cut the thyroid into small bits. Dry at 100° for 24 hours, obtaining a rough moisture determination at the same time. Grind the resulting glassy mass and again determine moisture at 100°. Extract the fat with anhydrous ether in an apparatus in which the sample is surrounded by vapors of the boiling solvent. Weigh 0.1 gram of powdered extract into a $2 \times 5 \times 1$ cm. nickel boat with one end cut away. Mix with about 2 volumes of freshly ignited calcium oxide, using a platinum wire. Cover completely with a thin layer of lime.

The apparatus for combustion is a modification of that used for foodstuffs (page 730). The combustion tube of Pyrex is 210×24 mm. The outlet tube is 155×5 mm Replace the absorption bulbs by 150×15 mm. test tubes containing 0.1 per cent sodium sulfite solution. Use two

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[6] Roe E. Remington, J. F. McClendon, Harry von Kolnitz and F. Bartow Culp, *J. Am. Chem. Soc.* 52, 980-5 (1930).

of these in parallel, splitting the gas stream with a Y tube. As an inlet use 3-mm. capillary tubes further drawn out to a fine tip. Cool the tubes in ice water. Unite the gas stream with another Y after passing these and pass through a 235 × 10 mm. vertical soda-lime tube. Put a glass filter disc in the bottom of this and fill with glass wool moistened with 0.1 per cent sodium sulfite solution. Connect the soda-lime tube to a suction flask connected to a pump. Fit the rubber tube connecting to the suction flask with a clamp to regulate the rate at which gas is drawn through. For heating use a torch operating on illuminating gas and oxygen.

Insert the boat in the combustion tube about 4 mm. from the inner end. Regulate the flow of air. Ignite the torch to give a nonluminous flame, oxygen being in excess. Heat, starting at the far end of the boat. After the first burning let the boat cool and mix the contents with a platinum wire. Again ignite in the tube to burn out any black spots. Repeat if necessary. The absorbers should be clear and colorless. Fat must be absent from the sample as it will distill onto the tube.

Rinse receivers and combustion tube into a 250-ml. beaker. Add the boat and residue. Rinse the tube first with ethanol, then with water, and add the rinsings. The washings should not exceed 225 ml. Evaporate to about 50 ml. Centrifuge to remove insoluble lime and evaporate to a small volume. Transfer to a nickel or platinum boat and evaporate to dryness.

Sprinkle about 0.5 gram of powdered sodium hydroxide over the residue and place in a combustion tube such as was previously used. Heat by a flame underneath until completely fused. Draw air through and use only one absorption bulb. This is no more trouble than heating in an open dish and avoids overheating and loss of iodine. Careful heating without this apparatus can be substituted.

Allow the flux to cool and dissolve in 10 ml. of water. Transfer to a 50-ml. beaker. To this add the rinsings of the tube and the contents of the absorption bulb. Add about 5 mg. of sodium azide. This removes any nitrites formed by combustion of proteins. Make just acid to bromophenol blue paper with 85 per cent orthophosphoric acid by testing small drops on the paper outside of the solution. Add 0.5 ml. excess of the phosphoric acid and 0.25 ml. of 8 per cent sodium sulfite solution.

Heat until the odor of hydrazoic acid disappears. Transfer to a 50-ml. volumetric flask and dilute to volume. Transfer 10 ml. to a separatory funnel for determination by solvent extraction. If the iodine content is very low, evaporate to 5-8 ml. before acidifying, dilute to 10 ml. when acid, and extract the entire sample.

Oily Seeds, Nuts, and Dry Milk. Pack in a sausage casing and handle as described for foodstuffs (page 730). The casing takes the place of the dry stick. Proceed, starting with "Advance this slowly into a silica tube . . ." If the sample is of dried milk, it is desirable to add cerium oxide or thorium oxide as a combustion catalyst.

Cereal Grains.[7] Mosten the sample with 50 ml. of 2 per cent sodium carbonate solution. This will result in a high fusing-point alkaline ash. Dry and grind to coarse granules. Heat in an evaporating dish over a small flame until it begins to smolder. Withdraw the heat and let burn without flame. Ignite again if necessary, giving a black char at last. Heat in a muffle furnace at not over 450° to give a light gray ash. A white ash is impractical as 12-15 hours will be required for the gray stage. Extract and proceed as for foodstuffs (page 730), beginning "Then add dropwise a mixture of 9 parts . . ."

Eggs.[8] Place the liquid contents of the eggs in a flask. For each egg add an equal volume of 95 per cent ethanol and 10 grams of potassium hydroxide. Reflux for 24 hours. No foaming or bumping occurs and the product is a dark brown liquid, nearly free of solids. This converts organic iodine into iodide ion. Transfer an amount of the liquid equivalent to 1 egg to a 500-ml. nickel crucible, or, if that is not available, to a beaker. Evaporate to dryness. Place in a muffle furnace and ash for 4 hours at 600°.

Extract the ash with 50 ml. of hot water. Filter and wash the residue with hot water. Acidify the filtrate and washings with 1:2 sulfuric acid until acid to methyl red. Add 5 drops more of acid and dilute for use of aliquots for the determination of iodine by solvent extraction.

Kelp.[9] Shake 5 grams of comminuted sample with 150 ml. of water for 3 minutes and dilute volumetrically to 250 ml. for use of filtered 25-ml. aliquots.

Isolation of Iodine. As a general technic to avoid possible loss by decomposition and volatilization of potassium iodide in combustion methods, use hot acid for decomposition and distill the resulting iodine or hydriodic acid.

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[7] J. F. McLendon and Roe E. Remington, *J. Am. Chem. Soc.* 51, 394-9 (1929).
[8] H. J. Almquist and J. W. Givens, *Ind. Eng. Chem., Anal. Ed.* 5, 254 (1933).
[9] W. N. Aldridge, *Analyst* 70, 474 (1945).

must be removed from ethanol by reduction as follows: Add 0.5 gram of iodine crystals to 500 ml. of absolute ethanol. Let stand for 24 hours and distill. Discard the first 25 ml. and leave 50 ml. in the flask. Shake the distillate with 100 grams of zinc until it is no longer yellow. Distill as previously described. Add 100 grams of granulated zinc and shake for 15 minutes. Distill as before.

Similarly, to purify carbon tetrachloride or chloroform add bromine-water until the color persists. Remove excess bromine by several extractions with 0.1 per cent sodium carbonate solution. Wash with water, filter, distill, and reject the first fraction.

Procedure. *Mild Oxidation.* To the sample add 10 per cent of its volume of carbon tetrachloride or chloroform and shake. A pink color at this stage indicates the presence of iodate as well as iodide. If present, add 1 drop of 0.1 N arsenious acid and let stand 10 minutes to reduce. Add 1 drop of 10 per cent potassium nitrite and shake for 2 minutes to extract iodine. Centrifuge to clarify the solvent solution of iodine.

To insure the complete liberation of iodine withdraw 0.1 ml. of the aqueous layer, add 1 drop of chloroform or carbon tetrachloride and a small crystal of potassium iodide. Unless this solution becomes pink, add a drop of concentrated sulfuric acid to the main solution and repeat the extraction.

Withdraw the solvent solution and compare with a solution of standard similarly prepared.

Drastic Oxidation. Add saturated bromine-water to the acid sample until a permanent and definite yellow color is obtained. Boil until excess bromine is removed and the solution has been concentrated to a small volume. Cool and transfer the iodic acid solution to a small separatory funnel. Wash any crystals which have separated and add the washings.

Add a crystal of potassium iodide. Extract the liberated iodine with five 1 ml. portions of pure carbon tetrachloride. Compare the combined extracts with a standard.

Iodine in Aqueous Solution

Relatively large concentrations of iodides are determined by the brown color of free iodine liberated in acid solution by a few drops of bromine-water.[10] Bromides and chlorides do not interfere. If the amount of

[10] R. G. Turner, *J. Am. Chem. Soc.* **52**, 2768-73 (1930); *J. Biol. Chem.* **88**, 497-511 (1930).

iodide is relatively small, oxidize to iodate by boiling with excess bromine-water. Then 6 times the original amount of iodine is liberated from potassium iodide in acid solution by the reaction $HIO_3 + 5HI \rightarrow 3H_2O + 3I_2$. This may be estimated to 0.0005-0.005 mg. in the sample by its reaction with starch paste.

Procedure. *Direct Liberation by Bromine-water.* Prepare bromine-water by dissolving 2 ml. of bromine in 200 ml. of concentrated hydrochloric acid. Dilute 20 ml. of this with water to 1 liter for use. Take 50 ml. of sample, suitably diluted if necessary, in a comparison tube. Add 10 ml. of 1:1 sulfuric acid and bromine-water dropwise until a maximum color is produced. Compare with 50 ml. of standard from which the color has been similarly liberated.

Oxidation with Bromine-water. Dilute or evaporate an aliquot of sample containing about 0.001 mg. of iodine in a Pyrex test tube to 2 ml. Take a standard in a similar tube. Add 2 drops of 1:18 sulfuric acid and 3 drops of freshly saturated bromine-water. Let stand for one-half minute and boil off excess bromine over a micro burner. Cautiously evaporate to 0.5 ml. While hot, add 1 drop of 1 per cent salicylic acid solution and cool in water.

To prepare a starch solution, mix 0.5 gram of soluble potato starch with 2.5 ml. of water and work to a thin paste. Pour this gradually with stirring into 200 ml. of water. Heat to boiling and boil for 15 minutes, stirring constantly. When cool, add 0.25 gram of salicylic acid and stir until dissolved. It should be clear, require no filtering, and be used within 2 weeks.

Add 5 drops of starch solution to sample and standard. Add 3 drops of 1 per cent potassium iodide solution to each and dilute to 1 ml. Compare in a micro colorimeter after suitable dilution. The color is stable for 1 hour, increases slightly in 16 hours, and is not affected by temperature in the range of 10-60°.

Iodine by o-Tolidine

In neutral solution iodine gives with o-tolidine a blue-green color similar to that for chlorine. Iodides and iodates are determined by this color reaction after reduction of the iodates to iodides, and oxidation of the iodides to free iodine. Nitrites give the same color reaction and must be changed to nitrates which do not interfere. Smaller amounts of chlorine or bromine than 1500 ppm. do not affect the color under the

to 100 ml. For determination add 0.5 ml. of this to a 20-ml. sample containing 1-3 mg. of potassium iodate, and to a corresponding standard. Mix well and set aside for 5 minutes before reading.

Minute amounts of iodine are determinable by their catalytic effect on the reduction of ceric sulfate by arsenite.[15] The property is a linear function.

[15] Hidehiro Goto and Emiko Sudo, *J. Chem. Soc. Japan* 63, 1324-8 (1942).

CHAPTER 54

FLUORIDE

FLUORIDE is not widely distributed in quantity. Like so many chemicals, development of uses has nevertheless led to isolation in tonnage. Therefore occasion for detection of small losses from units using fluoride catalytically is one, but only one, of the applications. The amount in water supplies and foods is closely related to the deterioration and discoloration of dental enamel.

The methods are usually quite indirect, by displacement of a color-producing ion from a complex such as oxidized titanium solution, ferric thiocyanate, or a lake such as that of alizarin and zirconium. A number of such methods have been developed.

SAMPLES

General Methods of Isolation. *Liquids.* If the sample is alkaline, add a few drops of phenolphthalein solution to a known volume and add 1:100 hydrochloric or 1:150 nitric acid until acid. Boil and continue to add acid until the pink color no longer returns on further boiling. Add 0.4 per cent sodium hydroxide solution until just alkaline and let cool in a stoppered tube. When cool, again render just acid and add 1 drop of the acid used. Dilute to a known volume.

Solids. Figure 27 illustrates the evolution apparatus. Joints B and C are of ground glass, D is an adapter. Figure 28 shows the complete apparatus. Tube B is of rubber and carries compressed air. The gas wash-bottle D contains concentrated sulfuric acid. Four tubes E are 25 × 100 mm. and are filled with calcium chloride. Tube F is of similar size and contains phosphorus pentoxide and glass beads. Glass wool fills G. Tubes H and M are of rubber, controlled by clamps I and N. The receiver L is made from a 25 × 200 mm. test tube. Pressure is regulated by O. Trap P is connected to a water pump. The apparatus is so suspended that K can be shaken.

For use, oven-dry the evolution apparatus shown in Figure 27. Mix the sample with 2 grams of powdered glass in the bowl of the apparatus. If more than 3 grams of sample are used, increase the glass to 3 grams.

temperature, otherwise manganese may be extracted and give the solution a yellow color. While still moderately hot, drop the crucible with its contents into 200 ml. of water. Digest on a sand bath for 2-3 hours. Remove the crucible when all the material is disintegrated and concentrate the liquid to 70-100 ml. Cool, add 3-4 grams of ammonium carbonate, and let stand overnight. Filter and wash the residue with a 5 per cent ammonium carbonate solution. If there is any question as to complete extraction of fluorides, dry the residue and fuse again as a separate determination.

Evaporate the filtrate to dryness on a water bath. Take up with 50 ml. of water, filter, and evaporate to dryness. This removes silica, iron, and aluminum. Add 50 ml. of water saturated with phenolphthalein and titrate hot with 1:8 sulfuric acid. Do not let all the indicator color disappear or hydrofluoric acid may be lost. Boil after each addition of acid to expel carbon dioxide. When the end point is near, put the solution into a 100-ml. flask and complete the titration with 0.1 N acid. Evaporate the solution to 50 ml. Add 0.6 gram of powdered silver sulfate to the hot solution with stirring. Set aside overnight in a dark place and filter in the dark into a 100-ml. flask through double filter papers. Transfer the precipitate to the paper and wash carefully with 20 ml. of distilled water. Use the entire filtrate as a sample to maintain the salt concentration standard and determine by the effect on oxidized titanium solution.

Enamel. Fuse the sample with a suitable amount of sodium carbonate. Extract the melt with water and filter. Add an excess of ammonium carbonate to the filtrate and boil to precipitate silica. Filter and concentrate to a suitable volume. Neutralize with 1:1 sulfuric acid to phenolphthalein using the indicator on a spot plate. Use this or an aliquot as sample. If phosphates or other interfering substances are present, concentrate the fluoride by distillation (page 743). Determine preferably by the effect on an oxidized titanium solution.

Glass.[1] Fuse an exactly 1-gram sample with 3.5 ± 0.1 grams of sodium carbonate. Take up the melt in water, filter, preferably by suction, and wash with hot water. The majority of glass constituents remain behind as carbonates and are discarded. To remove the silica, alumina, and ferric iron remaining, add 20 ml. of a solution of 10 grams of zinc oxide in 200 ml. of 1:10 nitric acid. Again filter and wash the

[1] M. C. Parrish, J. H. Widmyer, and F. R. Matson, *Anal. Chem.* 19, 156-7 (1947).

residue with hot water. Use the filtrate for development with titanium and hydrogen peroxide.

Soil.[2] Fuse 5 grams of soil with 10 grams of a mixture of equal parts by weight of sodium carbonate and potassium carbonate. Dissolve the melt in water and evaporate to dryness on a water bath. Take up the melt with 75 per cent ethanol which dissolves all of the fluoride but relatively little of the carbonates and silicates. Filter and evaporate the filtrate to dryness. Take up with 95 per cent ethanol and filter. Evaporate to dryness and take up with water. Add 0.01 per cent silver sulfate solution carefully so that all chlorides, bromides and iodides are precipitated but no substantial excess is added. Filter and add an equal volume of 95 per cent ethanol to precipitate sodium sulfate. Filter and carefully add 1 per cent barium hydroxide solution until the solution is just alkaline to phenolphthalein. Filter and evaporate to dryness. Take up the residue with 1:100 hydrochloric acid. Filter if necessary and use as sample, or dilute to a known volume and use an aliquot.

Water. To 100 ml. of sample add 5 ml. of 2 per cent barium chloride solution. Let settle for several hours, filter if necessary, concentrate if desirable, and use for the determination of fluorides by zirconium nitrate and a hydroxyanthraquinone derivative.

Vegetable or Animal Matter. Mix the weighed sample with 10 per cent of slaked lime, using the minimum amount of water. Dry at 110° and grind to a fine powder. Calcine at 550-600° to remove organic matter. The ash should be definitely alkaline. Take up with water and carefully add 1:3 hydrochloric acid until effervescence ceases. Add 10 per cent potassium hydroxide solution until definitely alkaline. Add 0.3-0.4 gram of crystallized sodium sulfate and when completely in solution a slight excess of 10 per cent barium chloride solution. Not over 5 ml. should be required. Evaporate to dryness at 100°. Add sufficient cold water to redissolve the soluble salts and an equal volume of 95 per cent ethanol. Transfer to a centrifuge tube and wash with 65 per cent ethanol until the washings no longer give a test for chloride. The residue contains the fluoride together with sulfate, silicate, phosphate, borate and other salts of barium.

Plant Materials.[3] To a suitable aliquot in a crucible, add sufficient

[2] J. S. McHargue, *J. Assoc. Official Agr. Chem.* 18, 207-10 (1935).
[3] H. v. Zehmen, *Die Chemie* 57, 159 (1944).

vidual laboratory. This must be standardized as to volume of excess acid present, amount of sodium and potassium carbonates used and present in the final solution as sodium and potassium sulfates, and temperature of comparison, as well as in the volumes of the usual reagents added to develop the color. Properly standardized, the method will permit estimations down to 0.002 mg.

Procedure. A standard titanium solution is needed for this method. For the purpose follow the instructions for preparation of standard titanium solutions from potassium titanium fluoride (page 437) but use 3 grams. The resulting solution will approximate 1 mg. of titanium dioxide per ml.

To the sample containing 0.005-0.5 mg. of fluoride add 10 ml. of 1:2 sulfuric acid and 3 ml. of 3 per cent hydrogen peroxide. No brown color should appear. Add 10 ml. of titanium sulfate solution containing 1 mg. of titanium dioxide per ml. Dilute to 100 ml. Read in a colorimeter against a solution containing the same amounts of sulfuric acid, hydrogen peroxide, and titanium sulfate added to 50 ml. of water, then diluted to 100 ml. As this standard contains no fluorides, its reading is taken as representing 100 per cent color development.

Read the amount of fluorides from a curve determined empirically with known amounts.

Fluoride by Bleaching Ferric Thiocyanate

Ferric thiocyanate solution is changed from deep red to orange or yellow in the presence of fluorides. The color is inversely proportional to the amount of fluoride present and may be used for estimation of soluble fluorides in the absence of interfering substances. The change in color is due to the removal of iron by combination with fluoride. Sulfates and chlorides produce a small but similar effect. They must therefore be present only in small amounts or a correction made for them.

The alteration in color, although definite and reproducible, is not a straight line function. Caution must therefore be exercised in its application to apply all necessary corrections. Results by this method agree well with those by alizarin sodium sulfate.

Procedure. To the sample add 5 ml. of a ferric chloride solution made up by titration to contain 0.075 mg. of iron per ml. and containing 30 ml. of 1:11 hydrochloric acid per liter. Add the necessary excess of iron indicated by the curves, Figure 29, as necessary to be equivalent to

the sulfate and chloride present in the sample. Add 10 ml. of a 0.24 per cent solution of ammonium thiocyanate, dilute to 75 ml., and mix. Compare with a prepared curve which has been obtained with known amounts of fluorides.

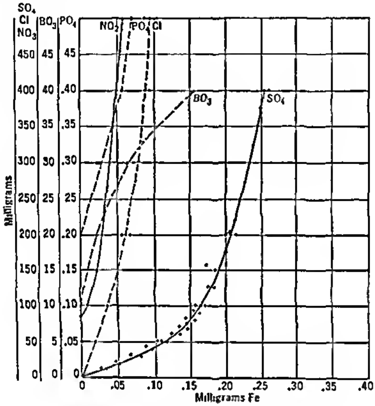

Fig. 29
Correction for NO_3, SO_4, Cl, BO_3, and PO_4 in Natural Water for Fluoride Determination

Fluoride by Bleaching Ferric Acetylacetone

As in the method for bleaching of ferric thiocyanate, the principle has been applied to the color of ferric salts with acetylacetone. The fading caused by different amounts of fluoride is a straight line function. An equilibrium condition rather than binding of iron by all the fluoride apparently occurs. The slope of the line is altered by acidity and other factors. It is therefore necessary to use as standard a portion of sample to which a known amount of fluoride has been added, comparing this and the sample against the same colored solution. By so doing errors due to variations in acidity and due to reasonable amounts of impurities are minimized

CHAPTER 55

SULFIDE

SULFIDE is often determined at levels where colorimetric methods are of superior accuracy. An example is the sulfide in contaminated air. Another is the sulfide from foodstuffs where it is indicative of the degree of decomposition. Yet another is the amount in manufactured gas. Methods include absorption and reading as a colored sulfide such as those of cadmium or arsenic, estimation by stains as on lead acetate paper, and conversion to sulfate for turbidimetric determination as barium sulfate. Carbon bisulfide is also determinable by other methods shown.

Gases.[1] Bubble the gas through a 6 per cent sodium hydroxide solution at the rate of 0.6 liter per minute until 0.006-0.040 mg. of sulfur is present. Mix and use an aliquot for the determination of sulfide as bismuth sulfide.

Acetylene. The oxidation of phosphine to phosphoric acid and hydrogen sulfide to sulfuric acid has been described (page 631). Use an aliquot for determination of sulfate by precipitation with an excess of barium chloride, precipitation of excess barium with chromate, and determination of excess chromate with diphenylcarbazide (page 275).

Carbon Bisulfide in Gases. Pass a suitable volume of gas through 10 per cent potassium hydroxide solution or other suitable gas absorbent in gas-wash bottles and then through concentrated sulfuric acid. It may be collected in a gasometer or the volume passed may be measured with a flowmeter. Then pass it through a capillary opening into 1 ml. of 10 per cent alcoholic potassium hydroxide solution in a test tube. After the desired volume of gas has passed, rinse the alcoholic solution of ethyl xanthate into a 50-ml. Nessler tube and determine bisulfide, preferably as copper xanthate.

Sewage and Mineral Waters. Select the sample to contain, if possi-

[1] Edmund Field and C. S. Oldach, *Ind. Eng. Chem., Anal. Ed.* 18, 665-7 (1946).

ble, about 8 mg. of sulfide and determine by *p*-aminodimethylaniline.

Feces. Add 5 per cent borax solution to the sample as a preservative until ready to use. For preparation grind the sample with 5 per cent borax solution in a ball mill to a uniform suspension. Dilute to a suitable volume with the 5 per cent borax solution. Transfer a suitable aliquot for determination of sulfide by *p*-aminodimethylaniline.

Foods. Aerate a finely chopped sample of suitable size for determination of sulfide by *p*-aminodimethylaniline. In general the sulfide concentration increases with the age of fish, beef, or pork.

Gelatin. This method is for labile sulfur only, that liberated by heating with concentrated ammonium hydroxide in the presence of silver ammonium chloride. Cut 5 grams of gelatin into pieces not over 0.6 cm. long and place in the sample container. Add 25 ml. of 1 per cent silver chloride solution prepared by solution of silver chloride in concentrated ammonium hydroxide. Let the gelatin swell for 1 hour and dissolve by heating slowly in a beaker of water to 50°. Continue to heat for about 2 hours. Too rapid heating will cause foaming. Shake at intervals to break the skin which forms on the surface.

In 2 hours the solution should be blackened due to evolution of sulfur and its fixation as silver sulfide. Evaporate to 10 ml. Cool the solution and transfer it to the evolution flask. For this sample the evolution apparatus should be run about 1 hour, as the silver sulfide is slow in decomposing.

Standard

Dissolve approximately 3 grams of sodium sulfide nonahydrate in 1 liter of 6 per cent sodium hydroxide solution. Determine the concentration by titration iodometrically. This should be freshly prepared daily. To prepare more dilute concentrations use 6 per cent sodium hydroxide solution as the diluent.

Sulfide as Lead Sulfate by Evolution as Hydrogen Sulfide

Sulfide sulfur is estimated by the stain which it produces on lead acetate-impregnated paper.[2] One such method uses an apparatus very

[2] Douglas V. Moses and Lawrence T. Jilk, U. S. Patent 2,232,622 (1941).

similar to that of the Gutzeit method for arsenic. The sulfur is evolved by reaction of a sulfur-free metal with hydrochloric acid and causes a stain of varying length on a prepared strip. Rigid standardization of the method of developing the stains is essential.

Sulfur-free zinc is not obtainable. Aluminum is the most satisfactory sulfur-free metal. Copper, nickel, and lead in the sample do not interfere. The method is applicable in amounts from 0.001 to 0.01 mg. Above 0.015 mg. the accuracy is impaired.

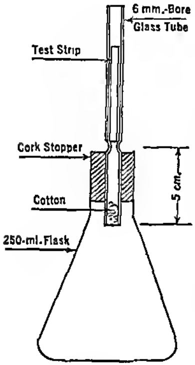

FIG. 30
Apparatus for Evolution of Sulfide With Hydrogen

Precautions to avoid sulfur in the ordinary distilled water and in reagents are essential. Samples and standards must be protected from light and air. The temperature must be sufficiently low so that drops of moisture will not condense in the parts of the apparatus containing the prepared paper.

Procedure. *Evolution with Hydrogen.* The evolution flask is shown in Figure 30. The lead acetate strip is contained in a 6-mm. tube having a slight constriction 5-mm. from the lower end. This tube is fitted to the flask with a cork stopper. The lower end of the tube carries a wisp of cotton to absorb acid spray.

To prepare the lead acetate paper, soak filter paper in 1 per cent lead acetate solution. Press out the excess between sheets of dry filter paper and hang in the dark to dry. When dry, cut the sheets into strips 5 mm. wide, of a convenient length, and store in stoppered tubes until required for use. Place the sample or an aliquot of a solution in 30 ml. of 0.5 per cent sodium hydroxide solution containing 0.1 gram of hydroquinone per liter. Heat just below boiling for 10 minutes. Cool to room temperature. Prepare aluminum reductor strips 1.5 × 3.0 cm. of stock 1.0-1.5 mm. thick. Clean just before use by immersing in boiling, 1 per cent hydrochloric acid, and rinse with distilled water. Add two reductor strips and 30 ml. of 1:2 hydrochloric acid to the sample. Stopper at once with the prepared dry tube containing a test strip. After reaction ceases, remove the strip and compare with those made to correspond to 0.001-0.01 mg. of sulfur.

As standard dissolve sodium thiosulfate in water and standardize at 0.1 N by titration with iodine. Dilute 10 ml. of this to 640 ml. Dilute 10 ml. of that solution to 1 liter. It is then equivalent to 0.001 mg. of sulfur per ml. Add 0.1 gram of hydroquinone and 5 grams of sodium hydroxide. Store in the dark. Prepare standards with 1-10 ml. of this at 1-ml. intervals. These correspond to 0.001-0.01 mg. Replace these at least every 2 weeks.

Sulfide as Arsenious Sulfide

Hydrogen sulfide coming into contact with paper impregnated with arsenious oxide forms a yellow stain of arsenious sulfide. This is particularly applicable to sulfur in basic and acid pig irons, as well as in steel. Checks to about 4 per cent with the volumetric and gravimetric methods were obtained in a laboratory doing about 100 samples a day.

Procedure. To prepare arsenious oxide paper shake 10 grams of pure powdered arsenious oxide with 30 ml. of concentrated hydrochloric acid. Add 500 ml. of boiling water and heat until dissolved. Dilute to 1 liter. Soak filter paper in this and drain over glass rods. Cut the paper while moist into pieces 10 cm. square and keep in a desiccator over

Introduce 1 gram of finely-ground sample or a corresponding v of solution into a flask. Add 10 ml. of benzine and 50 ml. of 7:3 chloric acid. Cover the vessel with moist arsenious oxide paper 10 square. Place on this a piece of white felt of the same size. Cover a piece of ebonite of the same size and weight down with a disc weighing about 500 grams. The hydrogen and hydrogen sulfide escape uniformly through the paper and produce a uniform tint. disturb after the evolution is started as breaking up the film of floating on the surface will cause irregular stains on the paper. At the same time run a similar determination with a co ing standard. Compare the stains by transmitted light.

Sulfide by p-Aminodimethylaniline

Sulfide sulfur which will be liberated by acid is absorbed acetate solution as a method of fixation. This is then p-aminodimethylaniline which reacts to give methylene blue sulfides such as methyl and ethyl mercaptans are not absorbed fore do not interfere. The method will detect 0.01 mg. of

Procedure. Prepare a zinc acetate solution by treating of glacial acetic acid with a slight excess of a thick aqueous

of zinc oxide. Dilute to 1 liter. This contains about 20 per cent of zinc acetate. Before use, filter to remove suspended solids and dilute 10 ml. to 100 ml. for a 2 per cent solution or 3 ml. to 100 ml. for a 0.6 per cent solution.

Fit a tall cylinder from which the hydrogen sulfide is to be evolved with a 3-hole rubber stopper. Fit a delivery tube connected to a carbon dioxide cylinder into one hole. This is also connected with a manometer filled with a zinc chloride solution of d. 2.0. The pressure registered is half that if water were used. Into a second hole put a dropping funnel containing 50 ml. of 1:1 hydrochloric acid. If the sample is organic use 50 per cent phosphoric acid instead. Into the third hold put an exit tube which also acts as a delivery tube for the hydrogen sulfide. This delivery tube extends beneath the surface of 30 ml. of a 0.6 per cent zinc acetate solution contained in a 100 ml. distilling flask used as the absorption vessel. Connect the side arm of this flask to a delivery tube extending beneath the surface of 20 ml. of a 0.6 per cent zinc acetate solution in a 100-ml. volumetric flask. This tube preferably contains many fine holes for efficiency in absorption. Rubber fittings used must be cleaned to free them from sulfides and free sulfur. They will then show no measurable blank.

Add the sample to 50 ml. of distilled water in the cylinder. Prevent foaming by addition of a few drops of diphenyl ether. In the case of egg products add 2 ml. of a 40 per cent solution of sodium tungstate. Connect the apparatus and sweep out the air with carbon dioxide. Let nearly all the acid flow into the cylinder from the dropping funnel. Pass carbon dioxide through at a pressure of about 40 mm. of water for 15 minutes. Shut off the carbon dioxide, disconnect the 2 receiving vessels, and transfer the solution from the distilling flask into the volumetric flask. The solution and washings should amount to about 90 ml. If the solution is turbid the sulfide concentration is usually greater than that of the highest standard. In that case dilute the sample to 100 ml., mix well and take an aliquot.

Add an aliquot of the sample distillate to 15 ml. of the 2 per cent zinc acetate solution in another 100 ml. volumetric flask and dilute to about 90 ml.

To the treated sample as above add 5 ml. of a fresh solution of 0.04 f p-aminodimethylaniline hydrochloride in 100 ml. of 1:1 hydro- acid. This must not be more than 24 hours old. Mix well and iol. of 0.02 M acid ferric chloride solution, tle shaking. is contains 27 grams of ferric chloride hexahyd l. of co centrated hydrochloric aci ed to a liter. se

with 4 volumes of distilled water. After 2 hours dilute to 100 ml. and compare in a colorimeter with a standard solution treated in the same way as the sample, having a color near that of the unknown.

Sulfide as Bismuth Sulfide

Absorption of sulfide and conversion to bismuth sulfide is a very sensitive technic.[3] Bismuth sulfide produces more sensitive changes than either cadmium or lead sulfide. The greatest sensitivity is obtained at the shorter wave lengths. Hence readings should be taken at the lowest available wave length, such as 350-355 mμ.

The precision is ± 10 per cent for 0.007 mg. of hydrogen sulfide, corresponding to 0.1 cubic foot of gas containing 0.11 grain of sulfur per 100 cubic feet or 0.0229 mg. per liter. With larger concentrations of sulfur the precision can be increased to ± 3 per cent. The presence of hydrogen, nitrogen, carbon monoxide, methane, ethylene and carbon dioxide up to 2 per cent do not interfere. Oxygen oxidizes sulfide ions and must be absent.

Procedure. To an aliquot of sample in 6 per cent sodium hydroxide solution add an equal volume of a reagent prepared as follows. Dissolve 4.28 grams of bismuth nitrate pentahydrate in 300 ml. of glacial acetic acid and dilute with 1.5 liters of water. In this weakly acid solution any carbonates present react to release carbon dioxide. Mix by bubbling nitrogen free from oxygen for 30 seconds, then allow to stand for exactly 5 minutes. Transfer a 10-ml. aliquot to a cuvette, read the transmittance, and compare with a calibration curve.

Sulfide by a Uranyl-Cadmium Reagent

The addition of a uranyl-cadmium reagent to a solution containing sulfide ions provides an effective means for estimating hydrogen sulfide colorimetrically.[4] Although not quite as accurate as determination with bismuth sulfide, it proves satisfactory where spectrographic equipment is not available.

Procedure. Prepare a blank made up of equal portions of 6 per cent sodium hydroxide solution, and a uranyl-cadmium reagent containing 4.41 grams of uranyl nitrate hexahydrate and 3.14 grams of cadmium

[3] Edmund Field and C. S. Oldach, *Ind. Eng. Chem., Anal. Ed.* 18, 665-7 (1946).
[4] *Ibid.*

acetate dihydrate in 2 liters of 1:5 acetic acid. The presence of the uranyl ion improves the accuracy in comparison work.

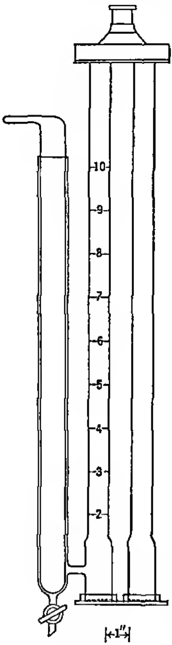

Fig. 31
Chromometer Comparison Tube

Dilute to 70 ml. an aliquot containing 0.03 0.200 mg. of sulfur in a 6 per cent solution sodium hydroxide. Mix by introducing a stre of nitrogen gas. Add 70 ml. of uranyl-cad reagent and again mix with a stream of nitroger Transfer about 120 ml. of blank solution to chromometer and the sample to a special mometer with a leveling tube and plunger, Fi ure 31. Allow to stand for 3 minutes. Adj the balancing plunger in the comparator until the color and intensity of the sulfide solu tion is the same as that in the blank. This is comparison by balancing.

Carbon Bisulfide as Copper Xanthate

Carbon bisulfide may be converted into alkali xanthate in alcoholic alkaline sol The color of copper xanthate formed from th is then suitable for colorimetric estimation about 10 per cent.

Procedure. As standard prepare a containing 0.0240 gram of ethyl xanthate liter of ethanol. Each ml. of this is equival to 1 mg. of carbon bisulfide. To 1-ml. portions of 10 per cent alcoholic potassium hydroxide solution add suitable volumes of standard. Dilute the standard and sample to about 48 ml. with distilled water. Add 2 drops of 0.01 per cent phenolphthalein solution and glacial acetic acid dropwise until faintly acid. Add 4 drops of 0.5 per cent cupric acetate solution, mix well and dilute to volume.

Miscellaneous

Hydrogen sulfide is determined in water by the brown color of colloidal lead sulfide, at the place where the sample is taken. The color of

the standard fades slowly due to oxidation of lead sulfide by dissolved oxygen. Samples containing hydrogen sulfide do not usually contain oxygen. The developed sample will therefore not ordinarily change on standing if protected from the air. Iron in small amounts does not interfere. If the sample is originally colored, treat with the reagent and let stand until the color of lead sulfide has entirely faded. Use for development of the standard color. Let the sample to be examined for hydrogen sulfide flow through a calibrated glass-stoppered flask of about 100 ml., until the air in the flask has all been displaced. Introduce into the bottom of the flask by means of a long-stemmed pipet, 5 ml. of a reagent containing 10 grams of sodium potassium tartrate, 10 grams of ammonium chloride and 0.1 gram of lead acetate per 100 ml. in 1:5 ammonium hydroxide. Stopper the flask and mix. Put 100 ml. of hydrogen sulfide-free water into a beaker and add 5 ml. of the lead reagent described. Add standard sulfide to this to match the sample.

In a mixture of sulfur dioxide and hydrogen sulfide, necessarily dilute as they would otherwise react, the sulfur dioxide is absorbed by neutral potassium chlorate solution, the hydrogen sulfide by hydrogen peroxide. Pass a known volume of gas through a wash bottle containing 5 per cent neutral potassium chlorate solution, then through a wash bottle containing 3 per cent hydrogen peroxide solution free from sulfates. Boil the peroxide solution to decompose excess peroxide, dilute to a volume which will give a proper sulfate concentration and estimate the sulfate turbidimetrically (page 768).

The color developed between ferric chloride and *p*-phenylenedimethyldiamine is reduced by hydrogen sulfide as a measure of the sulfide ion.[5] Sulfur in various forms in minerals and ores is oxidized to sulfate by fusion with sodium carbonate and sodium nitrate.[6] Continuous reading of the hydrogen sulfide content of gas is provided [7] by bringing the gas into contact with a molybdate-sulfite reagent and continuously recording the blue-green color photoelectrically.

The reaction of copper with sodium diethyldithiocarbamate is used for estimation of small amounts of carbon bisulfide in the presence of copper, since carbon bisulfide and diethylamine are the equivalent of the above reagent for copper. Thiophene, dimethylsulfide and ethyl mercaptan do not interfere, but thioacetic acid also reacts. Accuracy is good and 1 ppm. of carbon bisulfide can be detected. The method is equally applicable to toluene, carbon tetrachloride, acetone, ether and

[5] I. N. Markova and S. M. Gutman, *Zavodskaya Lab.* 12, 878-9 (1946).
[6] I. P. Alimarin and A. Ya. Sheskol'skaya, *Zhur. Anal. Khim.* 1, 166-75 (1946).
[7] Wilton E. Stackhouse U. S. Patent 2,413,261 (1946).

ethanol. In aqueous solution a precipitate is formed. As standard, dissolve 1 gram of redistilled carbon bisulfide in redistilled benzene and dilute to 100 ml. Dilute 1 ml. of this to 100 ml. as a standard containing 0.1 mg. of carbon bisulfide per ml.

Dilute the sample and standard with absolute ethanol to 0.025-0.1 mg. of carbon bisulfide per ml. Pipet 1 ml. of the sample into a cylinder. Add 1 ml. of 1 per cent diethylamine in benzene or other miscible solvent. Add 1 ml. of 0.03 per cent copper acetate solution in absolute ethanol. Dilute to 10 ml. with absolute ethanol and mix. Compare with a series of standards containing 0.025, 0.050, 0.075 and 0.1 mg. of carbon bisulfide. For accurate estimation make up a new sample and standard to match at the same time and compare after 20 minutes.

CHAPTER 56

SULFUR IN VARIOUS OXIDIZED FORMS

THE OXIDIZED forms of sulfur are as the lower oxide, sulfur dioxide, and the higher oxide which in solution is sulfate. Sulfur and sulfide may also be oxidized to sulfate and so determined. The usual method is turbidimetrically as the sulfate, with nephelometry following as a poor second. Sulfur dioxide is determined by a modified fuchsin reagent. The methods are largely old and well established, although occasionally variations are developed. There are also various indirect methods.

SAMPLES

Ferrous Alloys.[1] To determine the sulfur in ferrous alloys as sulfate, burn a suitable sample in a combustion tube, passing the gases evolved through an absorber containing glass beads or chips moistened with hydrogen peroxide, then through a solution of hydrogen peroxide, which has been prepared as follows: Add 0.2 ml. of 0.04 per cent sodium hydroxide to 20 ml. of freshly boiled conductivity water and 1 drop of methyl red solution. Add 10 drops of 30 per cent hydrogen peroxide and 50 ml. of conductivity water until the neutral point is reached.

Evaporate the sample solution on a steam bath to 1 ml., add 0 5 gram of pure copper oxide, and evaporate the remaining liquid. Dissolve the residue of copper sulfate in water, filter, and use an aliquot for the determination of sulfur indirectly by estimation of the copper with diethyldithiocarbamate. The sensitivity permits estimation of 0.002 mg. For detailed discussion of this general method of determination of copper see page 107. Alternatively determine the sulfur present, which will be as sulfide, by evolution as hydrogen sulfide.

Free Sulfur in Minerals.[2] Extract a weighed sample of the mineral with pyridine. To the solution so obtained, add a 1 per cent aqueous solution of casein dropwise until no further cloud appears. Determine nephelometrically as compared with standards.

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[1] G. Ingram, *Analyst* **70**, 423-6 (1945).

[2] Louis Peyron, *Compt. rend.* **222**, 740-1 (1946).

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Evaporate the sample solution on a steam bath to 1 ml., add 0.5 gram of pure copper oxide, and evaporate the remaining liquid. Dissolve the residue of copper sulfate in water, filter, and use an aliquot for the determination of sulfur indirectly by estimation of the copper with diethyldithiocarbamate. The sensitivity permits estimation of 0.002 mg. For detailed discussion of this general method of determination of copper see page 107. Alternatively determine the sulfur present, which will be as sulfide, by evolution as hydrogen sulfide.

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[1] G. Ingram, *Analyst* 70, 423-6 (1945).
[2] Louis Peyron, *Compt. rend.* 222, 740-1 (1946).

solution. Use as a sample for turbidimetric determination as barium sulfate.

Total Sulfate. Place 1 ml. of diluted urine in a Pyrex tube with 4 ml. of 1:11 hydrochloric acid and boil gently for 15-20 minutes. Discontinue heating when solid particles appear. Dissolve in 15 ml. of 1:100 hydrochloric acid. Add 2 ml. of 2.4 per cent sodium hydroxide solution and use as a sample for turbidimetric estimation as barium sulfate.

Alternatively, to 1 ml. of diluted urine in a Pyrex tube add 1 ml. of zinc-nitrate oxidizing mixture (page 765) and evaporate to dryness over a free flame. Dissolve the residue in 2 ml. of 1:11 hydrochloric acid. Add 15 ml. of water and use as a sample for turbidimetric estimation as barium sulfate.

Sulfate as Ester. This is often called ethereal sulfate. Subtract inorganic sulfate from total sulfate.

Neutral Sulfur. Subtract the total sulfate from the total sulfur.

Food and Biological Materials. Transfer a 2-gram sample to a 300-ml. Kjeldahl flask. Add 12 ml. of fuming nitric acid of d. 1.50 and allow the sample to dissolve at room temperature. After reaction appears to cease, warm gently until no more brown fumes are evolved. Add 7 ml. of 70 per cent perchloric acid and boil until white fumes appear. The solution should now be colorless, or nearly so. If necessary, let cool, add 3 ml. of additional perchloric acid, and heat again. To the cool solution, add 25 ml. of a 12 per cent sodium chloride solution. When reaction has ceased, boil off the chlorine evolved. Add concentrated ammonium hydroxide until alkaline and evaporate to dryness. Ignite the residue at a low heat to decompose ammonium salts. Dissolve the residue in 25 ml. of water and 1 ml. of concentrated hydrochloric acid. Dilute to nearly 100 ml. and use as sample for the determination of sulfur turbidimetrically as suspended barium sulfate.

Plant Tissue. The sulfur as sulfate, calcium, magnesium, sodium, and potassium are isolated as solution C in determination of lead (page 31). Take an aliquot.

Fruit. *Sulfur Dioxide.*[6] Macerate 0.25 gram of sample with 3 ml. of

[6] W. Morton Grant, *Anal. Chem.* 19, 345-6 (1947).

1 per cent potassium hydroxide in 10 per cent ethanol. Transfer all or an appropriate aliquot to the side bulb of the apparatus[7] shown in Figure 32. Place a pellet of sodium hydroxide weighing roughly 0.05 gram in the condenser portion. Freeze the sample in the bulb portion by immersion in a mixture of dry ice and acetone. When frozen, add a drop of concentrated sulfuric acid. Evacuate the apparatus, seal it, and let it warm to room temperature. The extract will melt and react with the sulfuric acid. Refreeze the acidified sample and then immerse the condenser portion in dry ice-acetone mixture. As the sample now melts it will distill the volatile components without heating. When distillation is complete, let the distillate melt and in so doing dissolve the sodium hydroxide pellet. Defer opening the apparatus for 30 minutes to absorb traces of sulfur dioxide gas. Use the solution as sample for determination with fuchsin and formaldehyde.

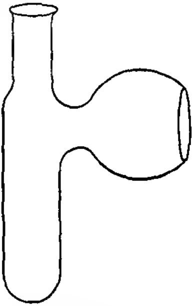

FIG. 32
Apparatus for Low-temperature Vacuum Distillation

STANDARD

Dissolve 0.5437 gram of potassium sulfate in water and dilute to 1 liter. Each ml. is equivalent to 0.1 mg. of sulfur as sulfate. Some of the indirect methods call for special standards which are included with the method.

As standard for sulfur dioxide, dissolve 1.5756 grams of anhydrous sodium sulfite, Na_2SO_3, in water and dilute to 1 liter. Each ml. is equivalent to 1 mg. of sulfur dioxide. For work of maximum accuracy standardize by iodometric titration of a portion.

SULFATES TURBIDIMETRICALLY AS BARIUM SULFATE

Sulfates, or sulfur oxidized to sulfate, may be determined by the turbid appearance of suspended barium sulfate. One method is conducted turbidimetrically by noting the height of the liquid in a cylinder just necessary to make invisible a flame underneath.[8] The various sulfur photometers can be used. Stabilizing agents such as peptone and gum

[7] W. Morton Grant, *Ind. Eng. Chem., Anal. Ed.* 18, 729 (1946).
[8] J. I. D. Hinds, *J. Am. Chem. Soc.* 18, 661 (1896).

gbatti are sometimes used. The turhidity of the sample may also be compared witb that of a series of standards precipitated under similar conditions.

The conditions under which the barium sulfate is precipitated must be carefully standardized. The most consistent results are obtained with 1-gram barium chloride tablets pressed together without the use of a binder. Barium chloride crystals, sized to 20-30 mesh,[9] and barium chloride solution are also used. The most common applications are to the estimation of sulfur as sulfate in bomb washings and to sulfate in water.

The tube used is similar in form to a Nessler tube, with the depth calibrated in cm. In the original design a candle is located about 2 inches below the tube. Variation in the size of the flame or its distance below the tube within reasonable limits does not affect the results. It is convenient to alter the form of the turbidimeter by use of a 1-candlepower electric bulb. Small amounts of nitrates do not interfere. A large excess of hydrochloric acid causes low results. Results are also read photometrically.[10]

Procedure. *Turbidimetrically.* Transfer the sample to a turbidimeter tube. Dilute to nearly 100 ml. and acidify with 1 ml. of 1:1 hydrochloric acid. Dilute to 100 ml., mix, and drop in a 1-gram barium chloride tablet or a standardized amount of barium chloride crystals. Close with a clean stopper. Tilt the tube up and down, causing the tablet to roll back and forth or the crystals to slide back and forth until completely dissolved. Avoid violent shaking. Transfer the liquid to a beaker. Light the candle or bulb and pour back a small quantity of the liquid into the turbidimeter tube. Put the tube in place and add more of the prepared suspension until the outline of the light just disappears when viewed through the column of still liquid. Pour back into the beaker, mix, and again read the height in centimeters. Determine the amount of sulfate present from Table 12.

Series of Standards. To a 10-ml. sample and to each of several 10-ml. portions of standard sulfate solutions add 1 ml. of an acid barium chloride solution containing 48 ml. of concentrated hydrochloric acid and 100 grams of barium chloride per liter. Mix well and compare. It is essential that standards and sample be treated at the same time.

[9] Soc. anon. des ciments de Thieu and L. Blondiau, Belgian Patent 448,926 (1943); Léon Blondiau, *Rev. matériaux construction trav. publ. C*, No. 380, 227-30 (1947).

[10] F. Blasco López-Rubio and J. de la Rubia Pacheco, *Ion* 5, 689-93 (1945).

Photometrically. To a 10-ml. aliquot of neutral, colorless sample, add in the cold, 1 ml. of 10 per cent barium chloride solution. Shake and read the transmittance of white light after a standardized time and compare with a table of results obtained under the same conditions.

Sulfate Nephelometrically as Barium Sulfate

The amount of sulfate present in a solution is estimated nephelometrically as barium sulfate. Rough comparisons can be obtained in test tubes or graduated cylinders. The amount of sulfur as sulfate per 25 ml. must be between 0.02 and 0.2 mg. for a satisfactory barium sulfate dispersion to be obtained.

Gelatin or glycerin is added to render the suspensions more stable. Limiting concentrations of other ions which may be present are sodium 0.15 per cent, magnesium 0.003 per cent, zinc 0.006 per cent, cadmium 0.006 per cent, mercury 0.04 per cent, and aluminum 0.002 per cent. Beyond these values magnesium gives low results, the others high results. Barium phosphate tends to precipitate unless the solution is properly acid. If too acid, barium sulfate precipitation is low. The optimum pH is 3.0-3.8. If nitrate is present, the results will be low. A uniform salt concentration in sample and standard is essential. Slight changes in conditions can vary results substantially.[11]

Procedure. To the prepared sample and standard add 5 ml. of a 1 per cent barium chloride solution, mix well and let stand for 15 minutes. Compare in a nephelometer.

Sulfate by Liberation of Chromate and Estimation with Diphenylcarbazide

By reaction of sulfate with barium chromate the corresponding amount of barium sulfate is precipitated and a soluble chromate left in solution. After removal of excess barium chromate by treatment with lime, the chromate is most satisfactorily estimated by its reddish violet color with diphenylcarbazide. One-tenth the amount which can be estimated by benzidine is thus determined. Phosphates react the same as sulfates and must be removed. By using an amount of barium chromate such that between 20 per cent and 80 per cent of it will react, the method is accurate to 2 per cent. Iron interferes, but in the presence of more than 0.17 mg. of iron per ml. of final solution this is avoided by addition

[11] E. Canals and A. Charra, *Bull. soc. chim.* 12, 89-91 (1945).

TABLE 12. TURBIDIMETRIC SULFUR TABLE. SULFUR AND SO_3 CONTAINED IN 100 ML. PRECIPITATED

Depth cm.	S mg.	SO_3 mg.	Depth cm.	S mg.	SO_3 mg.	Depth cm.	S mg.	SO_3 mg.
1.0	20.0	50.0	5.0	3.66	9.15	9.0	2.30	5.75
1.1	18.0	45.0	5.1	3.60	9.00	9.1	2.28	5.70
1.2	16.5	41.3	5.2	3.54	8.85	9.2	2.26	5.65
1.3	15.0	37.5	5.3	3.49	8.73	9.3	2.25	5.63
1.4	13.5	33.8	5.4	3.43	8.58	9.4	2.23	5.58
1.5	12.5	31.3	5.5	3.38	8.45	9.5	2.21	5.53
1.6	11.2	28.0	5.6	3.33	8.33	9.6	2.19	5.48
1.7	10.0	25.0	5.7	3.28	8.20	9.7	2.18	5.45
1.8	9.5	23.8	5.8	3.24	8.10	9.8	2.16	5.40
1.9	9.0	22.5	5.9	3.20	8.00	9.9	2.15	5.38
2.0	8.5	21.3	6.0	3.15	7.88	10.0	2.13	5.33
2.1	8.0	20.0	6.1	3.11	7.78	10.1	2.11	5.28
2.2	7.6	19.0	6.2	3.07	7.68	10.2	2.10	5.25
2.3	7.3	18.3	6.3	3.03	7.58	10.3	2.09	5.23
2.4	7.0	17.5	6.4	2.99	7.48	10.4	2.07	5.18
2.5	6.7	16.8	6.5	2.95	7.38	10.5	2.06	5.15
2.6	6.5	16.3	6.6	2.92	7.30	10.6	2.04	5.10
2.7	6.3	15.8	6.7	2.88	7.20	10.7	2.03	5.08
2.8	6.1	15.3	6.8	2.85	7.13	10.8	2.02	5.05
2.9	5.9	14.8	6.9	2.82	7.05	10.9	2.01	5.03
3.0	5.7	14.3	7.0	2.79	6.98	11.0	2.00	5.00
3.1	5.5	13.8	7.1	2.76	6.90	11.1	1.98	4.95
3.2	5.4	13.5	7.2	2.73	6.83	11.2	1.97	4.93
3.3	5.2	13.0	7.3	2.70	6.75	11.3	1.95	4.88
3.4	5.1	12.8	7.4	2.67	6.68	11.4	1.94	4.85
3.5	5.0	12.5	7.5	2.64	6.60	11.5	1.93	4.83
3.6	4.85	12.25	7.6	2.61	6.53	11.6	1.92	4.80
3.7	4.75	12.00	7.7	2.59	6.48	11.7	1.91	4.78
3.8	4.63	11.75	7.8	2.56	6.40	11.8	1.90	4.75
3.9	4.52	11.50	7.9	2.54	6.35	11.9	1.89	4.73
4.0	4.43	11.25	8.0	2.51	6.28	12.0	1.88	4.70
4.1	4.33	11.00	8.1	2.49	6.23	12.1	1.87	4.68
4.2	4.24	10.75	8.2	2.47	6.18	12.2	1.86	4.65
4.3	4.16	10.50	8.3	2.44	6.10	12.3	1.85	4.63
4.4	4.08	10.25	8.4	2.42	6.05	12.4	1.84	4.60
4.5	4.00	10.00	8.5	2.40	6.00	12.5	1.83	4.58
4.6	3.93	9.83	8.6	2.38	5.95	12.6	1.82	4.55
4.7	3.86	9.65	8.7	2.36	5.90	12.7	1.81	4.53
4.8	3.79	9.48	8.8	2.34	5.85	12.8	1.80	4.50
4.9	3.72	9.30	8.9	2.32	5.80	12.9	1.79	4.48
13.0	1.78	4.45	17.1	1.49	3.73	21.1	1.24	3.10
13.1	1.77	4.43	17.2	1.49	3.73	21.2	1.23	3.08
13.2	1.76	4.40	17.3	1.48	3.70	21.3	1.23	3.08
13.3	1.75	4.38	17.4	1.47	3.68	21.4	1.22	3.05
13.4	1.74	4.35	17.5	1.47	3.68	21.5	1.21	3.03
13.5	1.73	4.33	17.6	1.46	3.65	21.6	1.21	3.03
13.6	1.73	4.33	17.7	1.45	3.63	21.7	1.20	3.00
13.7	1.72	4.30	17.8	1.44	3.60	21.8	1.20	3.00
13.8	1.71	4.28	17.9	1.44	3.60	21.9	1.19	2.98
13.9	1.70	4.25	18.0	1.43	3.58	22.0	1.18	2.95

TURBIDIMETRIC SULPHUR TABLE—*Continued*

Depth cm.	S mg.	SO_4 mg.	Depth cm.	S mg.	SO_4 mg.	Depth cm.	S mg.	SO_4 mg.
14.0	1.70	4.2	18.1	1.43	3.58	22.1	1.18	2.95
14.1	1.69	4.23	18.2	1.42	3.55	22.2	1.17	2.93
14.2	1.68	4.20	18.3	1.41	3.53	22.3	1.16	2.90
14.3	1.67	4.18	18.4	1.41	3.53	22.4	1.16	2.90
14.4	1.66	4.15	18.5	1.40	3.50	22.5	1.15	2 88
14.5	1.66	4.15	18.6	1.40	3.50	22.6	1.15	2.88
14.6	1.65	4.13	18.7	1.39	3.48	22.7	1.14	2.85
14.7	1.64	4.10	18.8	1.38	3.45	22.8	1.13	2.83
14.8	1.63	4.08	18.9	1.38	3.45	22.9	1.13	2.83
14.9	1.62	4.05	19.0	1.37	3.43	23.0	1.12	2.80
15.0	1.62	4.05	19.1	1.37	3.43	23.1	1.11	2.78
15.1	1.61	4.03	19.2	1.36	3.40	23.2	1.11	2.78
15.2	1.60	4.00	19.3	1.35	3.38	23.3	1.10	2.75
15.3	1.60	4.00	19.4	1.35	3.38	23.4	1.09	2.73
15.4	1.59	3.98	19.5	1.34	3.35	23.5	1.08	2.70
15.5	1.59	3.98	19.6	1.34	3.35	23.6	1.08	2.70
15.6	1.58	3.95	19.7	1.33	3.33	23.7	1.07	2.68
15.7	1.57	3.93	19.8	1.32	3.30	23.8	1.06	2 65
15.8	1.57	3.93	19.9	1.32	3.30	23.9	1.05	2.63
15.9	1.56	3.90	20.0	1.31	3.28	24.0	1.05	2.63
16.0	1.56	3.90	20.1	1.30	3.25	24.1	1.04	2.60
16.1	1.55	3.88	20.2	1.30	3.25	24.2	1.03	2.58
16.2	1.54	3.85	20.3	1.29	3.23	24.3	1.03	2.58
16.3	1.54	3.85	20.4	1.28	3.20	24.4	1.02	2.55
16.4	1.53	3.83	20.5	1.28	3.20	24.5	1.02	2.55
16.5	1.53	3.83	20.6	1.27	3.18	24.6	1.01	2.53
16.6	1.52	3.80	20.7	1.26	3.15	24.7	1.01	2.53
16.7	1.52	3.80	20.8	1.26	3.15	24.8	1.00	2.50
16.8	1.51	3.78	20.9	1.25	3.13	24.9	1.00	2.50
16.9	1.50	3.75	21.0	1.25	3.13	25.0	1.00	2.50
17.0	1.50	3.75						

of 1 ml. of 20 per cent hydrochloric acid to the solution before developing the color.

Procedure. To prepare a diphenylcarbazide reagent, heat 6 grams of urea and 21.6 grams of phenylhydrazine for 45 minutes in a flask over a free flame. Let cool and break up the resulting mass. Extract excess phenylhydrazine with ether. Recrystallize the dried crystals from 300 ml. of boiling water. Filter and dry. Dissolve 2 grams of crystals in 10 ml. of glacial acetic acid and dilute to 100 ml. with 96 per cent ethanol. To 10 ml. of sample and standard add 1 ml. of the reagent and compare after 20 minutes.

As standard, use 0.5 ml. of 0.01 *N* barium chromate solution with 10.5 ml. of water. Each ml. of barium chromate solution is equivalent to 0.1603 gram of sulfur.

trioxide. To a second Nessler tube, add 1 ml. of the standard and 10 ml. of 0.5 per cent sodium hydroxide solution and dilute almost to the same volume as the sample. Add 1 ml. of the sulfide reagent, dilute to volume, and mix. Compare the sample and standard by balancing.

Sulfurous acid reduces phosphomolybdic acid to a blue color suitable for colorimetric estimation.[18] That combined with aldehydes or ketones is not liberated by addition of phosphoric acid and cannot be so estimated. Sugars, alcohols, organic acids, and salts do not interfere. Tannin and phenolic compounds also reduce the reagent and must be absent from the sample used.

In alcoholic solution ammonia liberates a violet form of sulfur from sulfur monochloride, which may then be used for its colorimetric estimation.[19] The method will detect 0.5 mg. of sulfur monochloride. To determine, pipet out 5 ml. of the sample of sulfur monochloride in carbon bisulfide, carbon tetrachloride, benzene, or similar nonaqueous solvent. Mix with 5 ml. of concentrated ammonium hydroxide and dilute to 100 ml. with 95 per cent ethanol. Compare with the color developed from a standard solution of sulfur monochloride in the same solvent. Free sulfur in a sample is dissolved in pyridine and then precipitated by the dropwise addition of a 1 per cent solution of casein for nephelometric determination.[20]

The sulfur in petroleum distillates[21] is estimated by the pink color of a modified Halphen test. Results agree with the ASTM lamp method for sulfur, and with amounts added. Hydrogen sulfide, mercaptans, sulfuric esters, organic sulfides, disulfides, sulfonic acids, and sulfones do not interfere. Excessive dilution causes low results. Therefore in no case should a sample be diluted to more than 50 per cent in excess of the original volume. If the sulfur content exceeds 0.15 per cent, a brownish precipitate will be formed which will not redissolve readily. High sulfur samples should therefore be diluted before treatment.

Place 20 ml. of the oil in an oil-sample bottle. As reagent mix 80 ml. of refined unbleached cottonseed oil, 80 ml. of freshly distilled carbon bisulfide and 8 ml. of pyridine. The reagent must be prepared fresh every day and should be stored in the dark in a glass-stoppered bottle.

For standards, purify naphtha by distillation over metallic sodium.

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[18] R. Sasaki, *Bull. Agr. Chem. Soc. Japan* **4**, 38-40 (1928).

[19] A. Castiglioni, *Ann. chim. applicata* **24**, 273-7 (1934).

[20] Louis Peyron, *Compt. rend.* **222**, 740-1 (1946).

[21] M. K. Thornton, Jr., and J. E. Latta, *Ind. Eng. Chem., Anal. Ed.* **4**, 441-2 (1932).

Dissolve 1 gram of sulfur in this naphtha and dilute to 100 grams. From this prepare dilutions containing 0.010, 0.025, 0.050, 0.075, and 0.100 gram of sulfur per 100 grams. The stock solution is not stable on standing and the dilute standards may deteriorate.

Add 4 ml. of reagent to sample and standards and heat for 30 minutes at 100°. Also run blanks of the sample and of the naphtha in which the standards are prepared. Cool and dilute each to 25 ml. with water-white gasoline. Compare, using the Walpole technique, Vol. 1, page 22, with the heated, untreated sample in front of the standard and the similar sample of purified naphtha in front of the sample. This corrects for natural color due to heating without reagent.

CHAPTER 57

SELENIUM AND TELLURIUM

SELENIUM and tellurium are relatively rare members of the sulfur family. As might be expected the metbods are limited in number and accuracy. Both are determined by metbods based on reduction to tbe element and estimation of the yellow to red color. A neutral or alkaline solution of selenic acid may be evaporated to dryness witbout loss, but witb balogen acid present the selenium will partially or completely volatilize.[1]

SAMPLES

Soil. A method bas been given under arsenic for separation of arsenic, selenium and germanium from interfering elements by distillation (page 186). Dissolve tbe selenium precipitate by adding 10 ml. of a solution of 1 gram of bromine in 10 ml. of concentrated bydrobromic acid. Catcb the solution in a 25-ml. volumetric flask and determine by reduction witb hydroxylamine.

Pyrites and Other Sulfides. Follow tbe details under arsenic (page 176) and finish as for soil, beginning, "Dissolva tbe selenium precipitate . . ."

Water. Follow tbe details under arsenic (page 177) and finisb as for soil, beginning, "Dissolve tbe selenium precipitate . . ."

Vegetable Matter. Follow the details under arsenic (page 181) and finish as for soil, beginning, "Dissolve tbe selenium precipitate . . ."

Tissue. Follow the details under arsenic (page 181) and finish as for soil, beginning, "Dissolve the selenium precipitate . . ."

Alternatively,[2] to 50-200 grams of comminuted sample add 50 ml. of concentrated sulfuric acid and 100 ml. of concentrated nitric acid. Heat on a water bath and transfer to a sand bath held at 120°. As soon as carbonization begins, remove from the bath. Transfer witb 4-5 ml.

[1] R. Dolique and S. Pérahia, *Bull. soo. chim.* 1946, 44-8

[2] R. Dolique, J. Giroux and S. Pérahia, *ibid.* 1946, 48-51.

of concentrated sulfuric acid, to a distilling flask and heat until white fumes are evolved. Continue to heat, and bubble hydrochloric acid gas into the mixture. Catch the distillate in 5 ml. of bromine-water in a large test tube contained in a water bath. Replace the receiver when the contents begin to get hot from dissolving hydrochloric acid. Combine all samples of distillate and add an additional 10 ml. of bromine-water to ensure that all selenium is in the quadrivalent form. Dilute to a suitable volume for use of aliquots, preferably by reduction with sodium metabisulfite.

Separation from Arsenic. For precipitation methods of separation see page 189.

Standard. Dissolve 0.1404 gram of selenium dioxide in water and dilute to 1 liter. This corresponds to 0.1 mg. of selenium per ml. If it is desired to express the results in terms of selenium dioxide, prepare the standard by dissolving 1.000 gram of selenium dioxide per liter. Similarly for tellurium use 0.1250 gram or 1 gram of tellurium dioxide.

Selenium by Reduction to the Element

Selenium in an acid solution, such as in hydrochloric acid, is reduced to the element with a suitable reagent, such as sodium metabisulfite, and the resulting orange to red color is read colorimetrically.[3] An analagous reaction is obtainable with stannous chloride as the reducing agent.[4] The color is stable for days and corresponds to Beer's law. Selenious acid is also reduced with sodium hyposulfite, $Na_2S_2O_4$, often called sodium hydrosulfite, for the determination of from 0.5 to 5.0 mg. of selenium dioxide in a 10-ml. sample. By concentration of a more dilute solution, as little as 0.06 mg. may be detected. To avoid production of free hyposulfurous acid which, in the absence of selenium, would give a false color, neutralize the solution with sodium carbonate after a few seconds. Reasonable amounts of neutral salts do not interfere. For comparison in concentrated sulfuric acid, the color is masked by the white of free sulfur precipitated. By suitable reduction of the amount of hyposulfite used the intensity of the yellow color indicates the amount of selenium present. An amount less than 0.02 mg. of selenium dioxide is not observable under these conditions. The color of reduced selenium can also be obtained by reduction with hydroxylamine.

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[3] R. Dolique, J. Giroux and S. Pérahia, *ibid.* 1946, 44 8.

[4] A. S. Shakhov, *Zavodskaya Lab.* 11, 893-5 (1945).

Procedure. *Reduction by Metabisulfite.* To an aliquot containing 0.1-1.0 mg. of selenium add sufficient concentrated hydrochloric acid to build up the acid concentration to 1:1. Add 1 ml. of glycerol to stabilize the solution. Reduce the solution by addition of excess of saturated metabisulfite solution and heat for 7 minutes at 70°. Compare with standards or read the transmittance with a blue filter and compare with a calibration curve.

Reduction by Stannous Chloride. Dilute an aliquot containing 0.05-0.5 mg. of selenium to about 25 ml. in a 50-ml. volumetric flask. Add 20 ml. of 1:1 hydrochloric acid, less an allowance for any already present. Add 1 ml. of 10 per cent stannous chloride solution and dilute to 50 ml. The reduction occurs without heating.

Reduction by Sodium Hyposulfite. If the sample is alkaline, render a 10-ml. portion containing 0.5-5.0 mg. of selenium faintly acid with dilute mineral acid. If strongly acid, make nearly neutral with sodium or ammonium hydroxide. Add 1 gram of dry sodium hyposulfite and shake. When the color ceases to become darker, add sufficient dry sodium carbonate to render the solution faintly alkaline and compare with a series of standards similarly treated at approximately the same time.

Reduction by Hydroxylamine. To the sample and to a series of standards add 1 ml. of a solution containing 5 per cent of gum arabic. Mix and pass in sulfur dioxide until the solution is decolorized. Add 0.5 gram of hydroxylamine hydrochloride and dissolve. Dilute to volume and let stand overnight. Shake the standards and sample and compare in sunlight.

Selenium by Potassium Iodide

The amount of selenious acid present in a solution may be estimated by the intensity of the yellow to brown color produced from its reaction with potassium iodide in acid solution. The use of starch to give a blue color does not give quantitative results. The method is applicable in the determination of 0.0001-0.05 per cent of selenium dioxide.

Procedure. Add a drop of 1 per cent gum arabic solution to the sample and standard mixed with 5 ml. of 1:1 hydrochloric acid. Mix and

dilute to about 90 ml. Add 5 ml. of fresh, colorless, 10 per cent potassium iodide solution and dilute to 100 ml. Mix and compare after 5 minutes.

Selenious Acid by Pyrrol

Pyrrol gives a deep blue coloration with selenious acid, suitable for colorimetric estimation. In phosphoric acid without addition of ferric ion it is sensitive to about 0.002 mg. per ml. By addition of iron this sensitivity is doubled. An alternative procedure is to replace the phosphoric acid by 1:1 sulfuric acid and ammonium molybdate to render it more sensitive but less specific. Silicic acid gives the same reaction. The color fades rather quickly.

Procedure. To 2 ml. of sample containing 0.005-0.05 mg. of selenium add 1 ml. of 5 per cent ferric chloride solution and dilute to 10 ml. with 85 per cent orthophosphoric acid. Add 10 drops of a 1 per cent solution of pyrrol in 95 per cent ethanol, mix, and compare with standards similarly treated at the same time. Alternatively, add 1 ml. of 5 per cent ferric chloride solution to 2 ml. of sample. Add 2 ml. of 8 per cent ammonium molybdate solution and dilute to 10 ml. with 1:1 sulfuric acid. Add 10 drops of a 1 per cent solution of pyrrol in 95 per cent ethanol. Mix and compare with standards similarly treated at the same time.

Selenium by Mercurous Chloride

Selenium is precipitated by mercurous chloride from a solution containing 6-20 per cent of hydrochloric acid. At least 16 per cent is necessary for complete reaction. At 20 per cent it is possible to detect 0.0002 mg. The color of the precipitate of excess mercurous chloride then serves for its colorimetric estimation. Arsenic, gold, platinum, palladium, tellurium, and iodine interfere but can be separated by precipitation before making strongly acid. For more details see page 533.

Procedure. Transfer 0.1 gram of mercurous chloride to a beaker with 1 ml. of water and 4 ml. of concentrated hydrochloric acid which has previously been boiled with mercurous chloride and left in contact with it for 24 hours. Add sufficient sample, which contains at least 20 per cent of hydrochloric acid, to form a definite color. Mix well for a few minutes and let stand. Compare with a series of standards.

The colors developed are as follows:

Mg. Selenium	*Color on Mercurous Chloride*
0.2	Salmon red, cold; bright red, warm
0.05	Salmon pink
0.005	Strong pinkish cream
0.002	Pink cream
0.0005	Light cream
0.0002	Faint cream

Tellurium by Reduction to the Element

Like selenium, tellurium is reduced to a colloidal dispersion of orange to red color.[5] The system corresponds to Beer's law below 10 mg. per liter.

Procedure. To an aliquot of sample containing 0.025-0.5 mg. of tellurium add water to about 25 ml. Add 20 ml. of 1:1 hydrochloric acid and 1 ml. of 10 per cent stannous chloride solution in 1:1 hydrochloric acid. Dilute to volume. Read through a blue filter and compare with a calibration curve.

Tellurium by Mercurous Chloride

Like selenium, tellurium can be precipitated by mercurous chloride.[6] Gold, platinum, palladium, selenium, and iodine interfere. The presence of 0.003 gram of copper or 0.002 gram of iron interferes. As with selenium the presence of hydrochloric acid is necessary. In 1:4 hydrochloric acid solution 0.0005 mg. of tellurium can be detected. For more details see page 533.

Procedure. Follow that for selenium (page 532), using a selenium-free sample. The colors developed are as follows:

Mg. Tellurium	*Color on Mercurous Chloride*
0.2	Grayish yellow, turns grayish brown when hot
0.05	Cream yellow
0.005	Light cream yellow
0.0005	Faint cream

[5] A. S. Shakhov, *Zavodskaya Lab.* **11**, 893-5 (1945).
[6] Gordon G. Pierson, *Ind. Eng. Chem., Anal. Ed.* **6**, 437-9 (1934).

CHAPTER 58

THIOCYANATES

THIOCYANATES play an important part in some physiological reactions. Hence colorimetric methods for the determination of small quantities of this ion are applied to biological samples of various types. The general method is to obtain the pink to red color of thiocyanate with a standardized amount of ferric iron, but other colored complexes are applicable.

SAMPLES

Blood,[1] Serum or Plasma. Dilute 40 ml. of oxalated blood or serum with 40 ml. of water and add 40 ml. of 20 per cent trichloroacetic acid. Mix to deproteinize and centrifuge. Transfer 90 ml. of clear centrifugate, corresponding to 30 ml. of blood, to another centrifuge tube and precipitate with 10 ml. of 17 per cent silver nitrate solution. Precipitation of thiocyanate is quantitative because of the other substances present, which are precipitated with silver. These help to carry down the thiocyanate. Let the tube stand for 1 hour and centrifuge. Remove the supernatant liquid. Wash the precipitate twice with water and once with 95 per cent ethanol.

Suspend the precipitate in 10-20 ml. of water and pass in hydrogen sulfide. Heat to boiling and break up the particles with a glass rod, continuing the hydrogen sulfide treatment until the precipitate is completely decomposed and the thiocyanate liberated. The precipitate which remains is composed of silver sulfide only. Remove the liquid from the residue and wash with water. Pass air through the combined solution and washings to remove hydrogen sulfide. Evaporate to about 3 ml., but no less, and use for the determination of thiocyanate, preferably as copper pyridine thiocyanate.

Alternatively,[2] mix equal volumes of serum or plasma and 10 per cent trichloroacetic acid. Stopper and shake well. Let stand for 10-15 minutes and filter. Use a 5-ml. aliquot of the filtrate as sample for determination as ferric thiocyanate.

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[1] Konrad Lang, *Biochem. Z.* 262, 14-19 (1933).

[2] M. Herbert Barker, *J. Am. Med. Assn.* 106, 762-7 (1936).

Urine. *Normal.* Normal, protein-free urine, which is not too highly colored, can be used directly. Slightly acidify 20 ml. of urine and precipitate in a centrifuge tube with 10 ml. of 17 per cent silver nitrate solution. Continue as for blood, beginning "Let the tube stand for 1 hour . . ."

Pathological. To 45 ml. of urine in a 50-ml. volumetric flask add 4 ml. of colloidal iron oxide and 3-4 drops of a saturated magnesium sulfate solution. Dilute to 50 ml. with colloidal iron oxide, mix and filter. Slightly acidify 20 ml. of filtrate, corresponding to 18 ml. of urine, precipitate in a centrifuge tube with 10 ml. of 17 per cent silver nitrate solution and continue as for blood, beginning "Let the tube stand for 1 hour . . ."

Feces. Rub up 10 grams of feces with water and transfer to a 100-ml. volumetric flask. Add 10 ml. of colloidal iron oxide and 1 ml. of saturated magnesium sulfate solution and dilute to volume. Shake and centrifuge. Precipitate 50 ml. of clear centrifugate with silver nitrate solution and continue as for blood, beginning "Let the tube stand for 1 hour . . ."

Saliva and Gastric Juice. Saliva must first be centrifuged in order to be able to measure it quantitatively. To 2-5 ml. of sample add 8 parts of water and 1 part of colloidal iron oxide. Shake and centrifuge. Precipitate an aliquot of clear centrifugate with 10 ml. of 17 per cent silver nitrate solution and continue as for blood, beginning "Let the tube stand for 1 hour . . ."

Bile. To 30 ml. or more of bile add 8 parts of water and 1 part of colloidal iron oxide. Mix and centrifuge to remove bile pigments. Take an aliquot amounting to 90 per cent of the centrifugate. Precipitate with 10 ml. of 17 per cent silver nitrate solution and continue as for blood, beginning "Let the tube stand for 1 hour . . ."

Cerebrospinal Fluid. Deproteinize 45 ml. of fluid with 5 ml. of 20 per cent trichloroacetic acid. Shake and filter. Precipitate 40 ml. of filtrate with 10 ml. of 17 per cent silver nitrate solution and continue as for blood, beginning "Let the tube stand for 1 hour . . ."

Standard

Dissolve 1 gram of potassium thiocyanate in 800 ml. of distilled water. Prepare a standard silver nitrate solution containing 2.9195 grams of

silver nitrate and 5 ml. of concentrated nitric acid made up to 1 liter. Titrate a 20-ml. aliquot of silver nitrate solution with potassium thiocyanate solution, using ferric ammonium sulfate as indicator. Calculate the amount of water to be added to the potassium thiocyanate solution to make 20 ml. equivalent to 20 ml. of the silver nitrate solution. Add the calculated amount of water, mix thoroughly, and check by a second titration against the silver nitrate solution. This solution should contain about 1 mg. of thiocyanate ion per ml. The standard is not too stable and should be frequently renewed.

Thiocyanate as Ferric Thiocyanate

When ferric nitrate or ferric chloride solution is added to a solution of thiocyanate, the conventional red color of ferric thiocyanate results, proportional to the concentration of thiocyanate.[3] Precautions should be taken against bleaching by sunlight. The technic can be applied to blood without necessitating precipitation of protein.[4]

Procedure. *Ferric Nitrate.* Prepare a ferric nitrate reagent by dissolving 50 grams of the salt in 500 ml. of water, add 25 ml. of concentrated nitric acid, and dilute to 1 liter. To 5 ml. of clear filtrate of sample add 1 ml. of ferric nitrate reagent. Mix and, after 5 minutes, read the transmittance at 550 mμ, using a reagent blank as reference. Compare with a calibration curve.

Ferric Chloride. To a 10-ml. aliquot add 0.5 ml. of 1:100 hydrochloric acid and 0.5 ml. of 1 per cent ferric chloride solution. Mix and compare with a standard solution of potassium thiocyanate similarly treated.

Thiocyanate as Copper Pyridine Thiocyanate

A solution of a copper salt reacts with pyridine and thiocyanate to form a blue or green complex compound, $Cu(C_5H_5N)_2(CNS)_2$, copper pyridine thiocyanate. This is used for the estimation of very small amounts of thiocyanate. The thiocyanate is first precipitated as the silver salt, the salt is decomposed with hydrogen sulfide, and the complex formed in the solution by the addition of copper sulfate and pyridine. The colored compound is extracted with an organic solvent. The reaction is specific. This procedure permits the determination of 0.025-0.2 mg.

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[3] M. Herbert Barker, *J. Am. Med. Assn.* **106**, 762-7 (1936); A. Colangiuli and A. Mastropaolo, *Diagnostica tec. lab.* (Napoli), *Riv. mensile* **12**, 17-26 (1941); Pietro De Franciscis, *Boll. soc. ital. biol. sper.* **22**, 779-81 (1946).

[4] W. N. Powell, *J. Lab. Clin. Med.* **30**, 1071-5 (1945).

of thiocyanic acid. The method has been applied to the determination of thiocyanate in pure solutions with an error of ±4 per cent. With blood and urine, recovery of added thiocyanate showed negative errors, the maximum for blood being −5 per cent, and for urine −8 per cent.

Procedure. Transfer the aliquot to a graduated glass-stoppered centrifuge cup of 10-ml. capacity. Wash the previous container until the washings bring the volume to 6 ml. Add 1 ml. of pyridine and 1 ml. of 10 per cent copper sulfate solution. A cornflower blue color appears. Add a further 0.5 ml. of copper sulfate solution. Extract 3 times with 1-ml. portions of bromobenzene, centrifuging after each addition. Siphon off the bromobenzene extract and filter through a small filter. If the amount of thiocyanate is high, further extraction may be necessary. Compare the united bromobenzene extracts with standard thiocyanate solutions, using a series of standards, each treated like the prepared sample solution. Beer's law does not hold for all concentrations.

THIOCYANATES AS BENZIDINE PYRIDINE THIOCYANATE

Thiocyanates, as well as cyanides, are converted to cyanogen bromide by bromide-water, and the resulting product reacts with a benzidine-pyridine mixture to give a deep orange to red color which may be used for colorimetric estimation.[5]

Procedure. To a 2-ml. aliquot which is neutral or slightly acid and which contains up to 0.004 mg. of thiocyanate add 0.2 ml. of bromine-water. Add 0.2 ml. of 2 per cent arsenious oxide solution to remove excess bromine. Prepare a constant boiling mixture of 57 ml. of pyridine and 43 ml. of water, boil, and cool. Add 3 ml. of this mixture and 0.6 ml. of a 5 per cent solution of benzidine in 2 per cent hydrochloric acid by volume. Mix and, after 15-20 minutes, read with a filter centering around 520 mμ and a heat-absorbing filter. The color is stable for 30 minutes at 20°.

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[5] W. N. Aldridge, *Analyst* 69, 262-5 (1944); *ibid.* 70, 474-5 (1945); J. L. Moglia, *Anales. farm. bioquím.* (Buenos Aires) 17, 18-32 (1946).

CHAPTER 59

NITRATE

NITRATE is important in soil as relating to fertilization, and in water as showing its past history of contamination. Oxides of nitrogen absorbed from the air are determined as nitrate. There are many industrial materials containing desired or contaminating amounts.

Nitrate and the nitrite of the next chapter are closely interrelated in the sense that the nitrate may be quantitatively reduced to nitrite and nitrite quantitatively oxidized to nitrate. Either may be reduced to ammonia. The best-known method for estimation as nitrate is by reaction with phenoldisulfonic acid, the standard method for analysis of water and sewage of the American Public Health Association. There are several other methods such as the reaction with diphenylamine or diphenylbenzidine, formation of nitrated xylenol, or the familiar qualitative reaction with ferrous sulfate in strong sulfuric acid.

SAMPLES

Air.[1] To determine nitric oxide collect atmospheric samples in a 2-liter evacuated glass bulb. Draw an aliqnot into another evacuated bulb and absorb the nitric oxide in either 10 ml. of 1:500 sulfuric acid or 10 ml. of 0.4 per cent sodium hydroxide solution, containing 3 drops of 30 per cent hydrogen peroxide. Refrigerate 2 hours to insure complete absorption of the gas and oxidation to the nitrate and use all or an aliquot for determination by the phenoldisulfonic acid method. Good recovery is obtained in the presence of carbon monoxide, ammonia, and hydrogen.

Alternatively,[2] pass a known large volume of air through a flask containing 10 ml. of 62.5 per cent sulfuric acid. Oxidize nitrous acid to nitric acid with 2 per cent potassium permanganate solution. Use 5 ml. of the solution for determination of nitrate by xylenol.

Another alternative[3] is suitable for collection of vapors of nitroglycerine, pentaerithritol tetranitrate, and ammonium nitrate, as well

[1] Jacob Cholak and Robert R. McNary, *J. Ind. Hyg. Toxicol.* 25, 354-60 (1943).
[2] Herman Yagoda and F. H. Goldman, *ibid.* 25, 440-4 (1943).
[3] *Ibid.*

as oxides of nitrogen. Draw at least 60 liters of air through 10 ml. of triethylene glycol. Propylene glycol is an acceptable substitute. Use 5 ml. of this sample for conversion to nitroxylenol and determination in that form.

Plating Solutions.[4] Prepare a precipitating solution to contain 13.5 grams of perchloric acid, 93.6 grams of silver perchlorate, and 258 grams of barium perchlorate per liter. To a 5-ml. sample in a beaker containing up to 120 grams of potassium nitrate per liter, add dropwise 1-ml. portions of the precipitating solution and mix. Permit the precipitate to settle 2-3 minutes after each addition. When no further precipitate forms, cover with a watch glass and allow to stand at least an hour. Filter and wash the precipitate 3-4 times with water. Use all or an aliquot for determination by a method appropriate to the impurities present. One method is to dilute the combined washings and filtrate to 50 ml. and read the nitrate content directly by the transmittance at 302-305 mμ. Cyanates, hydroxides, and carbonates do not interfere.

Soils. A method of preparation of sample has been given for the determination of potassium (page 545). Use an aliquot for reduction to ammonia.[5]

Less than 300 Mg. of Humus.[6] Transfer 5-10 grams of freshly sifted fine earth into a liter flask, add 10 ml. of water, and determine nitrates by the xylenol method, modified by first adding quickly 100 ml. of sulfuric acid-xylenol reagent (page 794). Then start at "Treat with four 15-ml. portions . . ."

High in Humus. To 50-100 grams of fresh sample add an equal volume of a 1 per cent solution of potassium aluminum sulfate dodecahydrate. Shake vigorously and frequently for 30 minutes and filter. To a 10-ml. aliquot of the filtrate add 100 ml. of sulfuric acid-xylenol reagent (page 794). If a yellow color does not appear immediately, cool to 20° and add 10-ml. aliquots of soil extract until a yellow color does appear. If more than 3 aliquots are added, increase the sulfuric acid content to maintain a 66 per cent sulfuric acid concentration.

[4] Albert Dolance and Paul W. Healy, *Ind. Eng. Chem., Anal. Ed.* 17, 718-19 (1945).

[5] R. F. Reitemeier, *ibid.* 15, 393-402 (1943).

[6] Cecil Treschow and E. K. Gabrielsen, *Z. Pflanzenernähr., Dungung Bodenk.* 32A, 357-76 (1936).

Continue as outlined in the first procedure (page 794), beginning "Treat with four 15-ml. portions . . ."

Chloride Content under 15 Ppm. Dry and mix the pulverized soil by passing through an 8-mesh sieve. If necessary, as with soil containing small hard granules, grind so that it will pass a 60-mesh sieve. This facilitates wetting and extraction. Put 50 grams of soil or 25 grams of peat in a wide-mouth bottle and shake for 10 minutes with 250 ml. of a solution containing 5 ml. of a 16 per cent copper sulfate solution. A water-soil ratio of 2:1 is as efficient in extraction of nitrates as that of 8:1. Let the precipitate settle and decant about 125 ml. Add 0.2 gram of calcium hydroxide and 0.5 gram of magnesium carbonate. Shake for 5 minutes to precipitate the copper and iron and filter through a coarse, dry filter paper. Discard the first 20 ml. Refilter, if necessary, through the same paper. A fine filter paper will retain sufficient nitrate to affect the results appreciably.

Take a 10-ml. aliquot of the treated and filtered sample if the nitrate content is more than 10 ppm. It should not exceed 50 ppm. If less than 10 ppm. take a 25-ml. aliquot. Determine by the phenoldisulfonic acid method.

Chloride Content 15-80 Ppm. Soils of humid regions seldom contain over 15 ppm. of chlorides. If the content of the sample is over that amount, add 10 ml. of a 0.4 per cent solution of silver sulfate. This is sufficient to remove up to 80 ppm. of chlorides in the original soil. Shake for 10 minutes, let settle, and decant 125 ml. Proceed as for solutions of lower chloride content, starting at "Add 0.2 gram of calcium hydroxide . . ."

Chloride Content over 80 Ppm. Add a sufficient amount of solid silver sulfate to the soil suspension before filtering so that all of the chloride will be precipitated. Shake for 10 minutes, let settle, and decant 125 ml. Excess silver is removed with copper and iron by the phenoldisulfonic acid method. Proceed as for solutions of low chloride content, starting at "Add 0.2 gram of calcium hydroxide . . ."

Strongly Colored Extracts. If the usual copper treatment is not sufficient, add 1 gram of high grade carbon black to each 100 ml. of supernatant liquid. Shake 15-20 minutes before adding calcium hydroxide. If necessary, add an additional 5 ml. of 16 per cent copper sulfate to assist in complete removal of the carbon black. It is to be expected

that some removal of nitrate will occur with this method and that the results will be low.

Alternatively, if soil extracts contain a large amount of soluble organic matter, they are decolorized by alumina cream. Shake 100 grams of soil and 5 grams of precipitated calcium carbonate with 400 ml. of distilled water for 15 minutes in a 1-liter bottle. Let settle and pipet off a 150-ml. aliquot. In a similar container put 150 ml. of distilled water. Add a 1 per cent solution of caramel, prepared as for matching the color of sugar solutions, until the color of this blank matches the sample. For each ml. of caramel solution used add the alumina cream from 0.75 gram of potassium aluminum sulfate to the sample. Shake thoroughly and filter through coarse filter paper. Proceed with aliquots, starting at "Add 0.2 gram of calcium hydroxide . . ."

For Determination by Pyrogallol. Shake 100 grams of soil for 1 hour with 200 ml. of distilled water. Filter and collect 80 ml. of filtrate. To this in a 100-ml. flask add 3 ml. of saturated barium hydroxide solution. Heat to boiling and let settle. Add 1 ml. of 50 per cent basic lead acetate solution. After 2-3 minutes add about 5 ml. of a saturated solution of sodium sulfate to remove excess lead and barium. Cool and dilute to 100 ml. Shake, filter, and collect 10 ml. of filtrate. This corresponds to 4 grams of sample.

If nitrites exceed 0.1 mg. of nitrogen trioxide per kg. of earth, add 1 drop of a saturated solution of urea and 1 ml. of concentrated sulfuric acid. Nitrites disappear within 10 minutes and no nitrates are formed.

Spent Nitration Acids.[7] As a suitable sample dilute 25 grams to 250 ml. in a volumetric flask and analyze at [illegible] air oxidation of nitrous acid giving high results.

Water.[8] If necess[illegible] [illegible]olorize with a[illegible] [illegible] as [illegible] in the next method [illegible] The [illegible] nitrite color should have b[illegible] [illegible]d. If [illegible] error will be intro[illegible] [illegible]it is remo[illegible] nitrates, add 4-5 [illegible] cent sol[illegible] free from nitrate to [illegible] heat, [illegible] times. As an altern[illegible] [illegible] oxidi[illegible]

[7] F. L. English, *Anal* [illegible]

[8] "Standard Metho[illegible] [illegible] Edition), pp. 69-71, A[illegible] [illegible] Assc[illegible]

potassium permanganate solution until a faint pink coloration persists. The value of the nitrites so oxidized to nitrates must be allowed for later.

If the nitrate nitrogen is less than 8 ppm., measure 50 ml. as sample. If very low, use 100 ml. If the nitrate nitrogen is more than 8 ppm., use only 1.0 ml. as sample. Add sufficient 0.02 N sulfuric acid to make the sample nearly neutral.

If chlorides are below 30 ppm., they need not be removed although they may cause up to 10 per cent error. If above that amount, add sufficient 0.4 per cent silver sulfate solution, equivalent to nearly 1 mg. of chloride per ml., to precipitate all but 0.1 mg. of chloride. Do not use an excess of silver sulfate. Filter and wash well with distilled water. Use as the sample for determination by the phenoldisulfonic acid method.

Sewage.[9] If the sample contains but little color, proceed as follows: Add 1 ml. of 10 per cent solution of zinc sulfate heptahydrate to 100 ml. of sample and mix. Add 0.5 ml. of 50 per cent sodium hydroxide solution, mix, and filter. Evaporate 1-25 ml. of filtrate, according to nitrate content, to dryness for determination by the phenoldisulfonic acid method.

To highly colored sewage add 0.5 gram of activated carbon to a 50-ml. sample and mix. Dissolve 125 grams of alum in a liter of water at 60° and add slowly, while stirring, 55 ml. of concentrated ammonium hydroxide. After an hour, transfer to an 8-liter bottle and wash the resulting alumina cream by decantation until free from chloride, ammonia, nitrite, and nitrate. Add 1 ml. of this alumina cream, mix, and filter. Discard the first portion of the filtrate. Evaporate 1-25 ml. of filtrate, according to nitrate content, to dryness for determination by the phenoldisulfonic acid method.

Vegetable Extracts.[10] If chlorides are present, add an excess of silver acetate and filter. To eliminate tannins, shake the sample with amyl alcohol and discard the alcohol extract. Place a suitable sample containing 0.01-0.05 mg. of nitrogen in a 50° water bath and evaporate to dryness in a vacuum. Use as sample for the determination of nitrates by the xylenol method.

Organic Samples. The method here described oxidizes nitrogen to nitrates and carbon to carbon dioxide. It is therefore applicable to deter-

[9] *Ibid.*, pp. 120-21.

[10] A. Hamy, *Ann. agron.* 15, 126-9 (1945).

mix. To 25 ml. of filtrate add 12.5 ml. of a 5 per cent solution of mercuric chloride and 12.5 ml. of 1:50 hydrochloric acid. Filter and dilute the filtrate to 500 ml. Use a suitable aliquot for determination by brucine.

Standard

Dissolve 0.7216 gram of dried potassium nitrate or 0.6068 gram of dried sodium nitrate in water and dilute to 1 liter. Each ml. contains 0.1 mg. of nitrogen as nitrate radical. If the report is to be in terms of nitrate radical, weigh 1.631 gram of potassium nitrate. Proceed with this solution as above. The resulting standard contains 0.1 mg. of nitrate radical per ml.

Nitrate by Phenoldisulfonic Acid

Nitrates in either hot or cold solution give a yellow color[11] with 1,2,4-phenoldisulfonic acid, prepared by treating phenol with fuming sulfuric acid. The color is intensified when the solution is made alkaline. The *ortho*- and *para*-phenolmonosulfonic acids react to give dark green solutions, the former reacting in the cold, the latter only when heated. The color compared is due to the formation of the tripotassium salt of nitrophenoldisulfonic acid, or by a modified procedure to the corresponding ammonium salt. Chlorides present up to 30 ppm. introduce an error amounting to not over 10 per cent and are not removed in some estimations, such as in water analysis. For the highest accuracy, chlorides, carbonates, organic matter, and large concentrations of nitrites must be absent. The reagent must be free from monoacids and must be of reproducible composition and concentration.

This method is used because of its simplicity, but has the objections of possible difficulty in obtaining a clear and colorless extract, possible loss of nitrates in evaporation, and occasional difficulty in comparison with the series of standards due to interfering tints. Loss is avoided by evaporation at a low temperature in alkaline solution. Accuracy to 5 per cent in comparison is to be expected.

Procedure. To prepare a phenoldisulfonic acid reagent, dissolve 25 grams of colorless phenol in 150 ml. of concentrated sulfuric acid. Traces of nitric acid present in the sulfuric acid can be removed by agitating

[11] Hermann Sprengels, *Ann. de Physik u. Chemie* 121, 188 (1864); "Standard Methods for the Examination of Water and Sewage" (Ninth Edition), pp. 69-71. American Public Health Association, New York, N. Y. (1946).

with mercury. Add 75 ml. of fuming sulfuric acid containing 13 per cent of free sulfur trioxide. Stir well and heat in a flask for 2 hours on a boiling water bath. The reagent so prepared is free from mono- and trisulfonic acids and may be heated in contact with a water residue for hours without development of interfering colors. Sulfonation is usually complete in 0.5-1 hour. Longer heating insures the absence of monosulfonic acids.

Make a sample containing 0.01-0.4 mg. of nitrate nitrogen alkaline and evaporate to dryness in a 3-inch porcelain evaporating dish. Add rapidly to the center of the residue of sample 2 ml. of the phenoldisulfonic acid reagent from a pipet or buret having the tip cut off. Rapid addition of an excess of phenoldisulfonic acid prevents loss of nitrates if considerable amounts of carbonates are present. Rotate the dish in such a way that the reagent comes in contact with all of the residue. After 10 minutes, add 15 ml. of cold water to each and stir with a glass rod until the residue is in solution. Heat if necessary to dissolve. When cool, slowly add 1.2 ammonium hydroxide until slightly alkaline. Ammonium hydroxide develops a better color and is less liable to give insoluble precipitates than potassium hydroxide. These precipitates are probably of magnesium. They may also be prevented from forming when potassium hydroxide is used for neutralization by addition of an ammonium salt.

Filter if necessary, transfer to a comparison tube, and dilute to 50 ml. Compare with a series of standards prepared to have the same volume. Correct the result obtained for any nitrite nitrogen oxidized to nitrate nitrogen, for the aliquot of the sample used, and for dilution of the aliquot.

Series of Standards. Evaporate to dryness on the water bath 50 ml. of a solution containing 0 1 mg. of nitrate nitrogen or nitrate radical per ml. Treat the residue with 2 ml. of phenoldisulfonic acid reagent in the same way as the sample. Dissolve in 15 ml. of water and dilute to 500 ml. Each ml. of this solution contains 0 01 mg. of nitrate nitrogen or nitrate radical according to the form of standard taken.

As standards, take 0.1, 0.3, 0.5, 0.7, 1.0, 3, 5, 10, 20, 30, and 40 ml. of the standard solution in which the color has been developed. These contain 0.001 to 0 4 mg. of nitrogen. Dilute each to about 40 ml. and add 1:2 ammonium hydroxide until faintly alkaline. Dilute to 50 ml. These standards will keep several weeks. The value of each standard in ppm. is 0 2 times the number of ml. of standard solution used.

APHA Method. The methods of the American Public Health Association call for the use of 67 per cent potassium hydroxide instead of ammonium hydroxide. Add 2 ml. to each standard instead of the ammonium hydroxide. In development of color of the sample add it until a maximum color is obtained. With that method of development of color, tripotassium nitrophenoldisulfonate, available in a pure form, may be used as standard. For such a standard dissolve 0.295 gram of the pure salt in water and dilute to 1 liter. Use this in the same way as the above standard. If protected from light, the colors will keep for years. To insure its accuracy, compare the standards so prepared with standards made from the standard potassium nitrate solution.

Nitrate by Xylenol

When 2,4-xylenol is added to a solution containing nitrates, a yellow nitration product forms which after treatment with dilute alkali may be used for the colorimetric estimation of nitrates.[12] Ammonia salts are not oxidized, and amino acids are split only in very small amounts. Chlorides and tannins interfere,[13] but the first is precipitated with silver acetate, the latter extracted with amyl alcohol, with no loss of nitrates in either case. For 0.05 mg. of nitrogen present, the error of the method is ±3 per cent, and this increases to ±10 per cent as the amount of nitrogen present decreases to 0.01 mg.

Procedure. *Aqueous Sample.* Make the sample solution alkaline and evaporate to dryness in vacuo at 50° in a flask of an all-glass distilling apparatus. Prepare a mixture of 25 ml. of 2:1 sulfuric acid and 0.1 ml. of a solution of 1.016 grams of xylenol in 5 ml. of acetic acid. Add to the dried residue of sample, stopper, and agitate. Allow to stand for 30 minutes at 20° and add 100 ml. of water. Add several small pieces of pumice stone and distill, collecting the distillate in a 100-ml. flask containing 25 ml. of 0.8 per cent sodium hydroxide solution. Make sure that no nitroxylenol remains in the condenser. Cool to 20°, add 100 ml. of 1:35 sulfuric acid, and mix. Treat with four 15-ml. portions of petroleum ether and combine the ether fractions. Wash the ether extracts with five 5-ml. portions of 0.8 per cent sodium hydroxide solution. Combine

[12] Jacob Blom and Cecil Treschow, *Z. Pflanzenernähr., Düngung u. Bodenk.* 13A, 159-90 (1929); Herman Yagoda and F. H. Goldman, *J. Ind. Hyg. Toxicol.* 25, 440-4 (1943). Francois L. Castillo Alzamoro, *Anales faculated farm. bioquim.* 5, 46-52 (1945).

[13] A. Hamy, *Ann. agron.* 15, 126 9 (1945).

the alkaline fractions and dilute to a suitable volume. Centrifuge if the solution is not clear. Compare with similarly prepared standards using a green filter, or read the transmittance and compare with a calibration curve.

Triethylene Glycol or Propylene Glycol Solution. To a 5-ml. aliquot in an all-glass distillation apparatus, add 5 ml. of water and 0.1 ml. of a 1 per cent solution of *m*-xylenol in the same solvent as the sample. Add dropwise 17 ml. of concentrated sulfuric acid to nitrate the xylenol. After 10 minutes, add an equal volume of cold water and distill by the preceding method, starting at "Add several small pieces of pumice stone . . ."

Nitrate by Reduction to Ammonia

Reduction of nitrates with Devarda's alloy, 45 per cent aluminum, 50 per cent copper, 5 per cent zinc, in a solution of sodium hydroxide permits the determination of the ammonia formed, by nesslerization. Aluminum is also used. Reduced iron and sulfuric acid give more consistent results with nitrates in bismuth carbonate. Nitrogenous organic compounds are hydrolyzed by distillation from a solution of sodium hydroxide, sodium carbonate, and magnesium oxide, buffered at 7.4. Other samples, such as tobacco, are oxidized by sulfuric acid followed by hydrogen peroxide.[14] Oxidation with perchloric acid is not advisable for organic samples since loss of nitrogen is difficult to avoid.[15] Should the method be preferred, care must be taken to use the barest excess of acid, since the loss of nitrogen increases with the increase of acid. Oxidation becomes more vigorous as the temperature increases. This has been variously explained as due to the formation of ammonium perchlorate and subsequent decomposition of the compound, to the partial oxidation of ammonium sulfate to free nitrogen, or to the formation of amines. Solutions containing chloride are best analyzed by this method rather than by the phenoldisulfonic acid method.[16]

Procedure. Dissolve 25 grams of sodium hydroxide in 125 ml. of water. Add several strips of aluminum foil weighing about 0.5 gram each and let stand overnight. Evaporate to about 100 ml. This elimi-

[14] A. S. Borozdina, *Vsesoyuz. Inst. Tabach. i Makhoroch. Prom.* No. 133, 166-71 (1937).

[15] L. F. Wicks and H. I. Firminger, *Ind. Eng. Chem., Anal. Ed.* 14, 760-2 (1942).

[16] R. F. Reitemeier, *ibid.* 15, 393-402 (1943).

nates any nitrates present as impurities. Add 2 ml. of the sodium hydroxide solution to a sample of 100 ml. or less containing 0.1-1.0 mg. of nitrate. Evaporate to about 20 ml. Transfer to a 100-ml. test tube with sufficient wash water to bring the volume to about 75 ml. Close the tube with a rubber stopper and bent exit tube which leads into a smaller test tube containing about 10 ml. of 1:350 sulfuric acid. This traps any ammonia evolved. Add 0.3 gram of pure iron or a strip of aluminum about $10 \times 6 \times 0.33$ mm. and put the stopper in place. Let stand overnight. Transfer the contents of both tubes to a distilling flask. Add 250 ml. of water and distill, collecting the distillate in a 200-ml. flask.

Determine the amount of ammonia in the distillate using the Nessler method (page 814).

Alternatively,[17] dissolve 70 mg. of selenium in 10 ml. of concentrated sulfuric acid. Cool the pale yellow solution thoroughly and add 200 ml. of cold 2:3 sulfuric acid. Add a suitable aliquot of sample and digest by placing the tube about a ¼ inch into the flame of the burner to prevent bumping. Transfer to a distilling flask and dilute to a suitable volume. Distill into a 200-ml. flask and determine ammonia in the distillate by the Nessler method (page 814).

Nitrate by Diphenylamine or Diphenylbenzidine in Sulfuric Acid

Nitrates produce a blue color with diphenylamine in sulfuric acid, which can be used for their estimation.[18] The colored product is a salt of diphenylbenzidine, produced by oxidation. Oxidation occurs in stages; diphenylamine is oxidized to diphenylbenzidine, both of which give colorless solutions, then diphenylbenzidine is oxidized to a blue salt. Both diphenylamine and diphenylbenzidine have been used to give the final product. Diphenylbenzidine gives twice the color intensity produced by diphenylamine with the same amount of nitrate. The color produced with diphenylbenzidine is a violet-blue rather than a clear blue, and is not as stable as the color produced with diphenylamine. Diphenylamine is therefore the more practical reagent, but diphenylbenzidine is used for smaller amounts of nitrate.

The conditions for the determination must be carefully controlled, as the color increases with increasing amounts of sulfuric acid and of chlorides, as well as of nitrates. The color decreases the lower the concentration of diphenylbenzidine. An increase in temperature increases the sensitivity of the reaction. The stability of the color depends on the

[17] Frederick Reis, *J. Lab. Clin. Med.* **29**, 666 (1944).
[18] E. Kopp, *Ber.* **5**, 284-5 (1872).

proportion of diphenylbenzidine to nitrate. A large excess of diphenylbenzidine must not be present. Because of the influence of so many factors, a series of reagents is used. The optimum ratio of sulfuric acid to water is 2:3. Since the reaction depends on oxidation, other oxidizing agents must be absent. From 0.05 to 5 mg. of nitrogen per liter of solution may be determined, using diphenylamine with the higher concentrations, and diphenylbenzidine with the lower.

Procedure. *Reagents 90/100 and 80/100.* Prepare a 90/100 solution by adding 100 mg. of diphenylamine to 900 ml. of water. Slowly add 1 liter of the concentrated sulfuric acid, prepared by boiling 1 liter with 2 grams of ammonium chloride at 180° for 1 hour, and cooling. Cool during the addition. To 6 grams of pure ammonium chloride in a storage bottle add 1 liter of the above solution. Prepare an 80/100 solution in the same way, except use 800 ml. of water.

To 1 drop of the sample solution on a spot plate, add 10 drops of the reagent. If a medium or dark blue color appears before 3 minutes have elapsed, dilute the sample at least 100-fold. If a pale blue color develops in less than 5 minutes, dilute 10 times. If a pale blue color develops in 5-10 minutes, dilute 1:1. If a blue color develops very rapidly, it is possible that nitrites are present. When enough nitrite is present to interfere, add to 10 ml. of sample 20 mg. of urea and 1 drop of concentrated sulfuric acid. Let stand overnight. Place 0.5 ml. of properly diluted sample solution in a small tube. Add 5 ml. of reagent slowly so that the temperature does not rise, but do not let the addition take over 20 minutes. Stir or shake until the solution is free from cloudiness. Place 0.5 ml. each of standard solutions containing 1, 2 and 5 mg. of nitrogen per liter in similar tubes and treat in the same way. After 1-3 hours, compare the sample with the closest standard.

Reagent 60/100. Prepare in the same way as the others, except use 50 mg. of diphenylamine, 600 ml. of water, and 3 grams of ammonium chloride in the storage bottle. Use in a manner similar to that described for the 90/100 reagent, except that mixing of solution and reagent must take place within 10 minutes. The standards used should contain 0.1, 0.2 and 0.5 mg. of nitrogen per liter.

Reagent 15/100. As reagent A, mix 100 ml. of water with 1 liter of the treated concentrated sulfuric acid. As reagent B add to 300 ml. of water and 200 mg. of diphenylbenzidine, 1 liter of the treated concentrated sulfuric acid. To 250 ml. of this solution add 0.2 gram of ammo-

nium chloride. Mix 0.5 ml. of sample solution with 1.5 ml. of reagent A and cool. Add 0.5 ml. of reagent B in not more than 10 minutes. Stir or shake once. Treat 0.5 ml. each of standard solutions containing 0.05, 0.1 and 0.2 mg. of nitrogen per liter in exactly the same way. Compare after 45 minutes.

Nitric and Nitroso-sulfuric Acids by Ferrous Sulfate and Sulfuric Acid

The need for determination of these acids arises in the handling of spent nitration acids. The reaction of ferrous sulfate and sulfuric acid with nitrate to give red ferrous nitrosyl sulfate, $FeSO_4 \cdot NO$, is suitable.[19] Such impurities as iron, lead, chlorides, copper, nickel, organic acids, etc., which may normally be present, do not interfere. The optimum color is developed in 5 parts of sulfuric acid to 1 part of water. A calibration as nitrite is suitable for reading the nitroso-sulfuric acid. The reaction is not proportional to the molar amounts of the acids present. Accuracy is to about 1 per cent.

Procedure. *Nitric and Nitroso-sulfuric Acids.* To a 2-ml. diluted sample add 1 ml. of water and chill in an ice bath. Add 1 ml. of 40 per cent ferrous ammonium sulfate solution in 20 per cent sulfuric acid with swirling. Similarly add 25 ml. of 5:1 sulfuric acid, slowly, over a period of more than a minute to avoid undue heating effects. Read the color within a half-hour using a green filter, the blank in this case being 5:1 sulfuric acid.

Nitric Acid. Mix a 2-ml. diluted sample with 1 ml. of 10 per cent sulfamic acid solution. Swirl to release all of the nitrogen bubbles and chill in an ice bath. Proceed as for mixed acids, starting at "Add 1 ml. of 40 per cent . . ."

Nitroso-sulfuric Acid. Determine by difference.

Miscellaneous

Nitrates are estimated by their color with brucine in the presence of sulfuric acid.[20] A suitable sample contains 0.01-0.2 mg. of nitrate nitrogen. The color is intense and persists for many hours. The estimation is

[19] F. L. English, *Anal. Chem.* 19, 850-2 (1947).
[20] L. W. Winkler, *Chem.-Ztg.* 23, 454 (1899); *ibid.* 25, 586-7 (1901).

made by the sulfur yellow that follows the initial red color, rather than by the original color which can not be relied on. If nitrites are not to appear in the final results, 2 parts of sulfuric acid must be present for every part of water. To determine nitrites as well as nitrates, lessen the amount of sulfuric acid so that the ratio of water:sulfuric acid is 2:1. If the sample solution contains much organic matter or ferrous iron, oxidize by the addition of 0.1 per cent of potassium permanganate solution until it is in slight excess. If this oxidation is carried out, nitrites will be oxidized to nitrates.

Put 10 ml. of sample in a 50-ml. volumetric flask and 10 ml. of distilled water in a similar flask. To the standard add a suitable volume of standard nitrate solution. To each add 0.2 ml. of a 5 per cent solution of brucine in chloroform and 20 ml. of concentrated sulfuric acid. After the color has changed to yellow, cool quickly and dilute each to 50 ml. with distilled water or sulfuric acid according to whether or not nitrite is to be determined. The colors may be compared any time within 24 hours.

Nitrate nitrogen reacts with reduced strychnine in the presence of sulfuric acid to give a rose-colored solution.[21] The reagent is sensitive to 0.01 ppm. of nitrate nitrogen. The method is so sensitive that many interferences due to color in the extract being examined can be eliminated by dilution. Peptone interferes by giving a purple color with sulfuric acid. Nitrites react with the reagent before the introduction of the acid, and should be determined, if present, and the reading subtracted from that for nitrate nitrogen. Oxidizing agents should be absent as they also give positive tests with the reagent. Chlorides do not interfere.

To prepare a strychnine reagent, mix 1 volume of a colorless 0.5 per cent solution of strychnine sulfate which is protected from air, in concentrated hydrochloric acid, with 1 volume of a 0.1 per cent solution of mercuric chloride in water. A 1 per cent solution of zinc chloride or a 0.002 per cent solution of lead chloride may be substituted for the mercuric chloride. Pour 25 ml. of the mixture cautiously over 1 gram of powdered magnesium in a 300-ml. flask. The reaction is very vigorous. Several flasks may be prepared and then combined. When cool, filter or decant. The reduced strychnine should be used within a few hours.

Introduce 1 ml of reagent into a test tube. To the sample tube add 5 ml. of the solution to be examined diluted to about 0.01 ppm. of nitrate nitrogen, then 5 ml. of concentrated sulfuric acid. Mix by pouring into

[21] G. Denigès, *Bull. soc. chim.* [4] 9, 544 (1911); F. M. Scales and A. P. Harrison, *Ind. Eng. Chem.* 16, 571-2 (1924).

another tube. Treat a series of standard volumes of nitrate solution in similar test tubes in the same way as quickly as possible. The full color development takes from a few minutes to one-half hour, the latter with very dilute solutions. The color deepens slowly for a long time and may still be compared after some hours if not exposed to light. A sample must not be compared with standards developed at a time more than 5 minutes earlier or later.

Pyrogallol sulfonic acid gives a rose color with nitrates or nitrites in the presence of sulfuric acid which after 1 hour will detect 0.00005 mg. of potassium nitrate per ml.[22] As the amount increases, the color is brownish red, olive green, and finally black. Pyrogallol gives similar color reactions but the test is less sensitive. Ferric ion gives a violet-red, iodates violet, chromates yellow, and organic matter brown. Small amounts of chlorates, ferrous ion, bromides, and chlorides do not interfere. Nitrites give the same colors as nitrates.

Prepare pyrogallol sulfonic acid by dissolving 5 grams of pyrogallol in 10 ml. of concentrated sulfuric acid. Heat for a few minutes at 80-90° until crystals form. Cool, dissolve in water, and dilute to 200 ml. Prepare a pyrogallol reagent by dissolving 5 grams of pyrogallol in water. Add 0.2 gram of sodium bisulfate and dilute to 200 ml.

To a sample low in nitrate in a 50-ml. porcelain dish add 0.5 ml. of pyrogallol sulfonic acid reagent. Mix and add 20 ml. of concentrated sulfuric acid. At the same time dilute different volumes of a standard nitrate solution to 10 ml. and treat each in the same way as the sample. These should cover the range of 0.00005-0.01 mg. of potassium nitrate per ml. Compare the sample after 1 hour with standards prepared at the same time. If the nitrate as potassium nitrate exceeds 0.01 mg. per ml., use 5 ml. of sample. Add 5 ml. of pyrogallol reagent and mix. Add 25 ml. of concentrated sulfuric acid and mix. Compare after 1 hour with a series of standards prepared at the same time.

Diphenylamine sulfonic acid is superior to diphenylamine as a reagent for estimation of nitrates.[23] Nitrites must be removed by boiling with ammonium chloride. Urea cannot be used for the purpose as it also interferes. Ferric iron also interferes.

To a 100-ml. sample containing nitrate, with no more than 1 mg. of nitrite, add 0.5 gram of ammonium chloride. Evaporate to 25 ml. to destroy the nitrites and dilute to 100 ml. Prepare a series of standard tubes containing 10 mg. of potassium chloride and 10 ml. of 0.1 0.2, 0.3,

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[22] Luigi Umberto de Nardo, *Compt. rend.* **188**, 563-5 (1929).

[23] I. M. Kolthoff and G. E. Noponen, *J. Am. Chem. Soc.* **55**, 1448-53 (1933)

0.4, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg. of nitrate per liter. Add about 10 grams per liter of potassium chloride to the sample and measure out 10 ml. To each tube add 10 ml. of concentrated sulfuric acid and stir while cooling in cold water. Then add 0.1 ml. of a 1.5 per cent solution of sodium diphenylamine sulfonate and mix. Compare as soon as the standard and comparable tubes on each side of it show sufficient color. There is no exact relationship between color and nitrate concentration. Therefore, for accuracy to 5 per cent repeat with a series of standards at closer intervals and a new sample.

The blue color with diphenylamine on a spot plate is used for a roughly quantitative estimation of nitrates, such as in field work.[24] The final concentration of sulfuric acid must be 70-90 per cent. Good results have been obtained with soil extracts and plant tissue.

Prepare a series of solutions containing 1, 2, 3, 5, 7, 10, 15, and 20 ppm. of nitrate nitrogen. Place 1 drop of each and 1 drop of the sample on a spot plate. Add 4 drops of a solution containing 0.05 gram of diphenylamine in 25 ml. of concentrated sulfuric acid to each of the drops. The solution should not be over 2-3 days old. Mix well and allow to stand. The full color develops in about 2 minutes. The nitrate nitrogen in the sample may then be estimated from the series of standards. A blank with distilled water must give no blue color. Variations cannot be readily observed above 25 ppm.

Nitric acid in sulfuric acid reacts with phenol to give picric acid. The resulting color is the basis of a method of estimation of small amounts of nitric acid in sulfuric acid.[25] To 20 ml. of a concentrated sulfuric acid solution of the sample, add 5 ml. of a mixture of 1 part of phenol, 4 parts of concentrated sulfuric acid free from oxides of nitrogen, and 2 parts of water. At the same time treat similarly 20 ml. of pure sulfuric acid to which a known amount of nitric acid has been added. Compare the yellow colors by dilution of the more deeply colored specimen with sulfuric acid known to be free from oxides of nitrogen.

Reduction of nitrate to nitrite by reaction with zinc is the basis of another method of determination.[26] Determination is then by α-naphthylamine and sulfanilic acid.

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[24] M. Francis Morgan, *Science* 61, 343-4 (1930).

[25] C. Berger, *Rev. gen. chim.* 14, 141-6 (1911).

[26] Erwin Haag and Charlotte Dalphin, *Arch. sci. phys. et nat.* 25, 148 (1943); Hideo Matsui, *J. Chem. Soc. Japan* 64, 809-10 (1943).

CHAPTER 60

NITRITES

Not only are nitrites of importauce as showing the presence of reducing agents, but nitrates may he quantitatively converted to nitrites for determination. For example, the nitrite content of water is a factor in the prediction of its past history.

The methods of determination generally depend on diazotization and coupling reactions. In acid solution, primary amines react with nitrites to form diazoninm salts which will couple to form various azo dyes. Sulfanilic acid and α-naphthylamine are the best-known reagents for this, but many others are used.

Samples

Water. If the sample is colorless, measure 100 ml. into a eylinder. If eolor present cannot be removed by simple filtration, decolorize as follows: Put 200 ml. of sample in a 250-ml. glass-stoppered hottle. Add 3 ml. of alumina cream (page 789) and shake. Filter after 15 minutes, discarding the first 25 ml., and determine the nitrate content by sulfanilie acid and α-naphthylamine.

Blood. Add exactly 8 ml. of freshly drawn blood to 20 ml. of a solution containing 4.5 per cent of zinc sulfate heptahydrate. The presence of sodium fluoride as an anticoagulant is immaterial. Prompt addition is necessary to prevent loss of nitrite. After mixing, this solution may stand for 24 hours without loss. Add 4 ml. of 4 per cent sodium hydroxide solution with thorough mixing and eentrifuge. Filtration will cause about 5 per cent loss of nitrite. Determine by α-naphthylamine and β-naphylamine-6,8-disulfonie aeid.

Standard

Sodium Nitrite. Dissolve 0 4926 gram of pure sodium nitrite in water and dilute to 1 liter. Dilute 100 ml. to 1 liter, and 10 ml. of the latter to 1 liter. Each ml. corresponds to 0.0001 mg. of nitrite nitrogen. This solution is stable when protected from hacteria and carbon dioxide. Add 1 ml. of chloroform to a liter of standard to prevent bacterial growth. The use of 0.05-0.10 gram of sodium hydroxide per liter pre-

vents the liberation of unstable nitrous oxide by any carbon dioxide that may be present.

NITRITES BY SULFANILIC ACID AND α-NAPHTHYLAMINE

These reagents are chemically 4-aminobenzenesulfonic acid and 1-aminonaphthalene. The reddish purple color developed when the sulfonic acid is acted on by a solution containing a trace of nitrous acid and the naphthylamine added for coupling may be used for estimation of the amount of nitrite present.[1] In the process the sulfanilic acid is converted by the nitrous acid into the corresponding diazo compound, and the latter reacts with the α-naphthylamine to form α-naphthylamine-*p*-azobenzene-*p*-sulfonic acid, a reddish purple azo dye. The full color which will result does not appear for several hours but if the temperature and other conditions of the standard and sample are identical the color will develop at the same rate.

High acidities increase the rate of diazotization and decrease the rate of coupling. Either acetic or hydrochloric acid may be used, but the latter is recommended. The color is more stable as the acidity increases. Increasing the temperature of the solution increases the reaction rate, but also decreases the stability of the diazotized salt. Ordinarily, the use of a sufficiently acid medium produces a diazotization reaction that progresses quite rapidly, even in a very cold solution.

The minimum transmittance is at 520 mμ. Beer's law holds for this method up to 0.6 ppm. of nitrite ion for a 10-mμ band width. Small amounts are determined by using thicker cells for measurement. Chromium up to 40 ppm., cobalt 100 ppm., oxalate 200 ppm., carbonate 200 ppm., chloroplatinate 80 ppm., chlorostannate 40 ppm., cyanide 100 ppm., dichromate 80 ppm., silicate 200 ppm., and tungstate up to 10 ppm. may be present.

This has also been adapted as a rapid field method, using a 50-ml. hypodermic needle for sampling, reaction and comparison,[2] and permanent standards.[3]

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[1] L. Ilosvay de N. Ilosva, *Bull. soc. chim.* [3] 2, 388 (1889); Herman A. Liebhafsky and Earl H. Winslow, *Ind. Eng. Chem., Anal. Ed.* 11, 189-90 (1939); Assoc. Official Agr. Chem., "Official and Tentative Methods of Analysis," p. 222, 527 (1940); B. F. Rider and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.* 18, 96-9 (1946); "Standards Methods for the Examination of Water and Sewage" (Ninth Edition), p. 72. American Public Health Association, New York, N. Y. (1946).

[2] F. A. Patty and G. M. Petty, *J. Ind. Hyg. Toxicol.* 25, 361-6 (1943).

[3] P. H. Averell, W. F. Hart, N. T. Woodberry and W. R. Bradley, *Anal. Chem.* 19, 59-60 (1947).

Procedure. Prepare a sulfanilic acid solution by dissolving 0.60 gram of recrystallized material in 70 ml. of hot water. Cool the solution, add 20 ml. of concentrated hydrochloric acid, and dilute to 100 ml. Mix thoroughly and, to an aliquot of sample containing 0.03 mg. or less of nitrite, add 1 ml. of the reagent. Mix well and allow to stand 3-10 minutes for diazotization at room temperature, preferably in the dark to prevent decomposition.

To prepare the α-naphthylamine hydrochloride reagent, use the pure colorless material. If not absolutely colorless, recrystallize 5-10 grams of the salt by adding 2 grams of decolorizing carbon and 100 ml. of water and boiling for 5-10 minutes. Filter rapidly through a Büchner funnel. If the filtrate is not clear and colorless, add another 2 grams of carbon, boil, and refilter. Add 25 ml. of concentrated hydrochloric acid to the filtrate and cool to 0° in an ice bath. Filter the crystallized mass on a Büchner funnel, air-dry on a porous plate in the dark, and transfer to a tightly closed dark bottle. Dissolve 0.60 gram of the salt in water containing 1 ml. of concentrated hydrochloric acid and dilute to 100 ml.

Add 1 ml. of α-naphthylamine hydrochloride reagent to the diazotized solution and buffer to pH 2.0-2.5 with about 1 ml. of a 20 per cent solution of sodium acetate. Dilute to 50 ml. and mix well. After 10 minutes measure the intensity of the reddish purple color at 520 mμ or compare with standards. As artificial standards for visual comparison, various dilutions of a 7 per cent solution of potassium dichromate are suitable.[4] Acid fuchsin may also be used.

Nitrites by Sulfanilamide and *N*-(1-naphthyl)ethylenediamine Hydrochloride

Nitrites are determined by the red color formed on the addition of sulfanilamide as a diazotizing agent and *N*-(1-naphthyl)ethylenediamine hydrochloride as a coupling agent.[5] The color is stable for 2-3 hours. The minimum transmittance is at 540 mμ.

Procedure. Dissolve 0.2 gram of sulfanilamide in 20 ml. of 1:4 hydrochloric acid. To a 0.5-ml. sample containing 0.00002-0.0005 mg. of

[4] E. Bohm, *Z. Untersuch. Lebensm.* 84, 403-15 (1942).

[5] A. Calvin Bratton, E. K. Marshall, Jr., D. Babbitt and A. R. Hendrickson, *J. Biol. Chem.* 128, 537-50 (1939); Martha B. Shinn, *Ind. Eng. Chem., Anal. Ed.* 13, 33-5 (1941); N. F. Kershaw and N. S. Chamberlin, *ibid.* 14, 312-13 (1942); W. J. Dyer, *J. Fisheries Research Board Can.* 6, 414-18 (1946).

nitrite as sodium nitrite per ml., add 1 ml. of the sulfanilamide reagent and mix. Add 1 ml. of a 0.02 per cent solution of *N*-(1-naphthyl)-ethylenediamine hydrochloride. Measure the transmittance, after allowing a few minutes for full development of color, and compare with a calibration curve.

Nitrites by Sulfanilic Acid and Dimethyl-α-Naphthylamine

The color produced with dimethyl-α-naphthylamine in reaction with diazotized sulfanilic acid is superior in intensity, stability and brilliance to that with α-naphthylamine.[6] The method is the same. The reagent is stable for at least 60 days. The effect of hydrogen sulfide is less than with α-naphthylamine.

Procedure. Prepare a sulfanilic acid-acetic acid mixture by dissolving 3.3 grams of sulfanilic acid in 750 ml. of water by the aid of heat and adding 250 ml. of glacial acetic acid. Add 10 ml. of this to the sample. Dissolve 5.25 grams of dimethyl-α-naphthylamine in 1 liter of 1:3 acetic acid prepared by dilution of glacial acetic acid with 95 per cent methanol. Add 10 ml. of this to the sample. Compare after 10 minutes with suitable standards treated similarly at the same time.

Miscellaneous

A very sensitive color reaction between α-naphthylamine hydrochloride, tartaric acid and nitrite is used for amounts of nitrite below 0.15 mg. per liter.[7] As reagent dissolve 5 grams of α-naphthylamine hydrochloride and 445 grams of tartaric acid in 50 grams of concentrated sulfuric acid. The reagent keeps indefinitely. Add 1 ml. of the reagent to 50 ml. of sample and a similar volume of a suitable standard. If necessary, aliquot the sample to lower the nitrite content to the limits of the method.

Bismark brown is formed by the action of nitrous acid on metaphenylenediamine reagent.[8] The method is preferably applied at a pH of 2.6-2.8. For the reaction, mix 2 ml. of 1:3 sulfuric acid and 1 ml. of a 5 per cent solution of metaphenylenediamine in 1:20 sulfuric acid. Add to 100 ml. of sample and compare with a series of standards.

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[6] G. I. Wallace and S. L. Neave, *J. Bact.* 14, 377-84 (1927); J. M. Gutierrez Diaz, *Arch. soc. biol., Montevideo* 10, 301-7 (1942).

[7] G. Romijn, *Chem. Weekblad* 11, 115 (1914).

[8] J. P. Griess, *Ber.* 11, 624 (1878); E. Bohm, *Z. Untersuch. Lebensm.* 84, 408-15 (1942).

Procedure. Prepare a sulfanilic acid solution by dissolving 0.60 gram of recrystallized material in 70 ml. of hot water. Cool the solution, add 20 ml. of concentrated hydrochloric acid, and dilute to 100 ml. Mix thoroughly and, to an aliquot of sample containing 0.03 mg. or less of nitrite, add 1 ml. of the reagent. Mix well and allow to stand 3-10 minutes for diazotization at room temperature, preferably in the dark to prevent decomposition.

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Add 1 ml. of α-naphthylamine hydrochloride reagent to the diazotized solution and buffer to pH 2.0-2.5 with about 1 ml. of a 20 per cent solution of sodium acetate. Dilute to 50 ml. and mix well. After 10 minutes measure the intensity of the reddish purple color at 520 mμ or compare with standards. As artificial standards for visual comparison, various dilutions of a 7 per cent solution of potassium dichromate are suitable.[4] Acid fuchsin may also be used.

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Procedure. Dissolve 0.2 gram of sulfanilamide in 20 ml. of 1:4 hydrochloric acid. To a 0.5-ml. sample containing 0.00002-0.0005 mg. of

[4] E. Bohm, *Z. Untersuch. Lebensm.* 84, 408-15 (1942).

[5] A. Calvin Bratton, E. K. Marshall, Jr., D. Babbitt and A. R. Hendrickson, *J. Biol. Chem.* 128, 537-50 (1939); Martha B. Shinn, *Ind. Eng. Chem., Anal. Ed.* 13, 33-5 (1941); N. F. Kershaw and N. S. Chamberlin, *ibid.* 14, 312-13 (1942); W. J. Dyer, *J. Fisheries Research Board Can.* 6, 414-18 (1946).

resulting solution is pale blue and opalescent. Add 10 ml. of glacial acetic acid. The solution is colorless or a faint pink. Mix 8 ml. of a clear solution of sample with 8 ml. of each of the reagents. At the same time treat a similar volume of a suitable standard in the same way. Heat the sample and standard in a water bath at 80° to develop the maximum color. At 38° this develops in 30 minutes, at 80° in 20 minutes. No fading occurs within 18 hours.

While diphenylamine in the presence of sulfuric acid reacts to give a blue color with all oxidizing agents as well as nitrites, hydrogen chloride in ethanol activates only the reaction with nitrite and gives a yellow to red coloration.[13] This is diphenylbenzidine quinoid hydrochloride. To a drop of the sample add 0.5 ml. of a 1 per cent solution of diphenylamine in ethanol and 5 ml. of ethanol saturated with gaseous hydrogen chloride. Heat and compare with a series of standards produced simultaneously.

[13] Julio Ludowieg, *Rev. centro estud. farm. y bioquím.* 1946/47, No. 1, 31-7.

Nitrous acid reacts with dimethylaniline to give the yellow color of *p*-nitrosodimethylaniline which is used for colorimetric estimation.[9] Nitrates do not interfere. The method will detect 1.0 ppm. As reagent, dissolve 8 grams of dimethylaniline in 100 ml. of 1:6 hydrochloric acid. Add 0.3 ml. of this and 0.1 ml. of concentrated hydrochloric acid to 100 ml. of sample. Prepare a standard at the same time. Compare sample and standard after 15-30 minutes.

An acetic acid solution of antipyrine gives a green color with nitrite.[10] The reaction will detect 50 ppm. Ferric salts or mineral acidity must be absent. Mix 5 ml. of sample and 5 ml. of a 1 per cent solution of antipyrine in 10 per cent acetic acid and compare with standards similarly treated at the same time.

The blue color produced by nitrites in acid solution by reaction with zinc iodide-starch solution is used as a basis of determination.[11] A color appears in 7 minutes if 0.00025 mg. of nitrite is present. The method is at best only roughly quantitative. Prepare the starch solution by boiling 5 grams of starch and 20 grams of stannous chloride in 100 ml. of water for several hours. Replace the water as it evaporates. To this solution add 2 grams of zinc iodide, dilute to 1 liter, and filter. Add 4 ml. of this solution to 50 ml. of the sample acidified with 2 ml. of 1:5 sulfuric acid. The blue color develops most rapidly in the light. Compare with standards developed at the same time in the same way.

Another sensitive reaction is obtained by coupling α-naphthylamine and β-naphthylamine-6,8-disulfonic acid[12] (Amino-G-acid), to give a violet-blue in acid solution. The reaction is so sensitive that it will detect 1 part in 750 million in a 10-cm. column. The reaction is not affected by nitrates, urea or uric acid, glycine, cystine, tyrosine, alanine or leucine in the concentrations normally encountered in blood. Because of the extreme sensitiveness of the reagent unusual care must be taken to avoid contamination of samples and reagents. Sample and standard must be very similar in concentration or the nature of the color is altered and sample and standard cannot be compared. With more than 0.07 mg. of nitrite per ml. the color becomes red. Prepare an α-naphthylamine reagent by boiling 0.1 gram of solid α-naphthylamine with 20 ml. of water. Decant the colorless solution for use. To prepare α-naphthylamine-6,8-disulfonic acid, add a small drop of the commercial 32.6 per cent solution of the sodium salt of Amino-G-acid to 50 ml. of water. The

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[9] E. H. Miller, *Analyst* 37, 345 (1912).
[10] M. C. Schuyten, *Chem.-Ztg.* 20, 722 (1896).
[11] E. A. Letts and F. W. Rea, *Analyst* 39, 350 (1914).
[12] Edward J. Stieglitz and Alice E. Palmer, *J. Pharmacol.* 51, 398-410 (1934).

and test with Nessler's reagent until the reaction for ammonia is negative. Add slowly to the sample 10 ml. of a 60 per cent solution of sodium hydroxide so that the acid and alkaline layers remain separate. Connect with the flask containing the solution of sample and mix. Distill and collect in a 50-ml. Nessler tube the condensed steam containing the liberated ammonia. Dilute to volume and determine nitrogen by nesslerization using 1 ml. of reagent. This method is not recommended for steels with a high silicon, titanium, vanadium, tungsten, or columbium content.

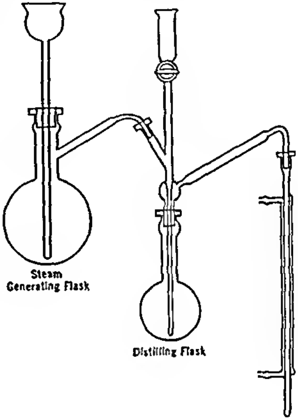

FIG. 34
Diagram of Micro Kjeldahl Unit

Soils. Ammonia nitrogen may be determined by nesslerization in an aliquot of sample prepared under potassium (page 545).

Total Nitrogen.[3] To a 0.3-2.0 gram sample, depending on the amount of organic matter present, add 5 ml. of concentrated sulfuric acid. Place on a hot plate to boil for 10 minutes, cool, and add dropwise 30 ml. of water and 20 ml. of 1.6 per cent potassium permanganate. Bail again for 10 minutes to oxidize all organic matter. Cool and add 1 ml. of a saturated solution of oxalic acid. If the supernatant liquid remains clear, oxidation is camplete. Distill the ammonia and determine by nesslerization.

Water-soluble Organic Compounds.[4] Digest 2 grams of sample in 100 ml. of water and filter. To the filtrate add 10 ml of 10 per cent ...uric acid and 10 ml. of a 2 per cent solution of potassium perman-...e. Heat to boiling for 5 minutes. Decompose excess potassium

[3] I. Levchenko, *Pedology (U.S.S.R.)* 32, 1489-93 (1937).
[4] ... Olendskil, *Vsesoyuz. Nauch. Issledovatel. Inst. Tabach. i Makhoroch. Prom.* ...17-57 (1937).

CHAPTER 61

AMMONIA

When nitrogenous materials of various types are digested, the ultimate form obtained is ammonia. For small amounts therefore the determination of ammonia is important. Similarly it is important in determining the past history of water, in that case involving ammonia in several forms. While it is established practice to distill the ammonia from many samples, direct digestion and nesslerization in that solution are feasible. An example is the preparation of blood samples. Another technic of value is removal by Permutit as illustrated by preparation of wine samples.

The important method is by reaction with Nessler's reagent, but a phenol-hypochlorite reagent is also occasionally used. Care must be exercised to have water and reagents ammonia-free. To prepare ammonia-free water, redistill 500 ml. of distilled water from a solution containing 1 gram of potassium permanganate and 1 gram of sodium carbonate. Reject the first 100-ml. portion of the distillate, after which distill about 300 ml.

Samples

Air.[1] Colorimetric results for ammonia in air are generally more accurate than those obtained by volumetric means. Wash a suitable volume of air by passage through gas wash-bottles and combine the washings as sample.

Steel.[2] *Combined Nitrogen.* Transfer to a distillation flask of a micro-Kjeldahl apparatus, Figure 34, 0.25-0.50 gram of sample containing 0.02-0.12 mg. of nitrogen, and cover with 10 ml. of 1:1 hydrochloric acid. Warm the flask to dissolve the sample Meanwhile, connect the steam flask and condenser to another distillation flask and apply heat to the steam flask so that a steady amount of steam passes over through the distilling flask to the condenser. Withdraw 50-ml. portions

[1] B. P. Utekhin, *Lab. Prakt.* (U.S.S.R.) 15, No. 11, 23-6 (1940).

[2] H. F. Beeghley, *Ind. Eng. Chem., Anal. Ed.* 14, 137-40 (1942).

and test with Nessler's reagent until the reaction for ammonia is negative. Add slowly to the sample 10 ml. of a 60 per cent solution of sodium hydroxide so that the acid and alkaline layers remain separate. Connect with the flask containing the solution of sample and mix. Distill and collect in a 50-ml. Nessler tube the condensed steam containing the liberated ammonia. Dilute to volume and determine nitrogen by nesslerization using 1 ml. of reagent. This method is not recommended for steels with a high silicon, titanium, vanadium, tungsten, or columbium content.

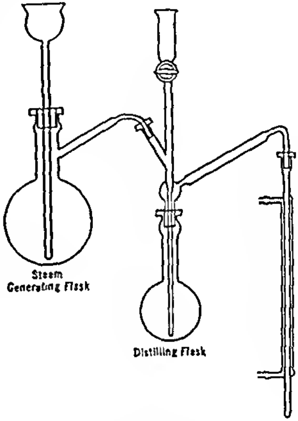

Fig. 34
Diagram of Micro Kjeldahl Unit

Soils. Ammonia nitrogen may be determined by nesslerization in an aliquot of sample prepared under potassium (page 545).

Total Nitrogen.[3] To a 0.3-2.0 gram sample, depending on the amount of organic matter present, add 5 ml. of concentrated sulfuric acid. Place on a hot plate to boil for 10 minutes, cool, and add dropwise 30 ml. of water and 20 ml. of 1.6 per cent potassium permanganate. Boil again for 10 minutes to oxidize all organic matter. Cool and add 1 ml of a saturated solution of oxalic acid. If the supernatant liquid remains clear, oxidation is complete. Distill the ammonia and determine by nesslerization.

Water-soluble Organic Compounds.[4] Digest 2 grams of sample in 100 ml. of water and filter. To the filtrate add 10 ml of 10 per cent sulfuric acid and 10 ml. of a 2 per cent solution of potassium permanganate. Heat to boiling for 5 minutes. Decompose excess potassium

[3] F. I. Levchenko, *Pedology* (U.S.S.R.) 32, 1459-93 (1937).
[4] V. I. Olendskii, *Vsesoyuz. Nauch. Issledovatel. Inst. Tabach. i Makhoroch. Prom.* No. 119, 47-57 (1935).

permanganate with a saturated solution of oxalic acid. Evaporate in vacuo to 1-2 ml. Distill and determine by nesslerization.

Water. *Total Nitrogen.* Evaporate 5 liters of filtered water to dryness on a hot plate. Dissolve the residue in distilled water to give 100 ml. of prepared solution. To 20 ml. of this solution add 5 ml. of concentrated sulfuric acid and 1 ml. of a 20 per cent solution of sulfosalicylic acid in 1:2.5 sulfuric acid and evaporate to dryness over an open flame. As soon as the residue chars, add 0.3 gram of pure iron and 4-5 ml. of water and allow to stand for 10-12 hours. To catalyze any reduction that is incomplete, add 0.1 gram of selenium, evaporate on a sand bath, and heat strongly for 20-30 minutes. Cool, add 1 ml. of 95 per cent ethanol, and heat again on the sand bath until the contents become colorless. Use an aliquot for the determination of nitrogen by nesslerization.

Ammoniacal Nitrogen. This is only the nitrogen originally present as ammonia or an ammonium salt. Set up an apparatus for distillation from a 1-liter distilling flask with a vertical block tin or aluminum condenser. Put distilled water in the flask and distill until the distillate shows no test for ammonia with Nessler's reagent. Empty the distilling flask. Place 500 ml. of sample or a smaller volume diluted to 500 ml. in the distilling flask. If acid to methyl orange, add 0.5 gram of sodium carbonate.

Distill at the rate of not less than 6 nor more than 10 ml. per minute, collecting the distillate in four 50-ml. portions in Nessler tubes. Use each as sample and total the nitrogen content found in the four. If the nitrogen content is high, use a 200-ml. calibrated flask as a receiver. In that case use as sample 50 ml., or an aliquot diluted to 50 ml., for development of color.

Alternatively, the ammoniacal nitrogen is obtained by direct determination. Put 50 ml. of sample, or an aliquot diluted to 50 ml. with ammonia-free water, in a glass-stoppered graduated cylinder. Add a few drops of 10 per cent copper sulfate solution and, if the original sample contains hydrogen sulfide, also add a few drops of 10 per cent lead acetate or zinc sulfate solution. Mix well and add 1 ml. of 50 per cent sodium or potassium hydroxide solution. Mix again, let stand for the precipitate to settle, pipet an aliquot of the clear upper layer, and use as sample. To prevent liberation of ammonia from amines and urea, hydrolyze in a solution buffered with borax to pH 7.6 and distill with steam at 70° under 80-100 mm. pressure.

Albuminoid Nitrogen. This is the nitrogen liberated as ammonia by the action of alkaline potassium permanganate after expulsion of ammoniacal nitrogen. To prepare the necessary permanganate solution, boil 1.2 liters of distilled water 10 minutes to drive off ammoniacal nitrogen. Add 16 grams of potassium permanganate and stir until dissolved. Add 800 ml. of clear 36 per cent sodium hydroxide or 50 per cent potassium hydroxide solution. Dilute to about 2500 ml. and concentrate to 2000 ml. by evaporating on a hot plate. Determine the ammonia in 50 ml. of the reagent and use as a blank.

Add 50 ml. or more of the alkaline potassium permanganate solution to the flask from which ammoniacal nitrogen has been distilled. Continue distilling until at least four 50-ml. portions have been collected. Use each as sample and total the results. The distillate may also be collected in a 250-ml. flask and 50 ml. used as sample.

Organic Nitrogen. This is all the nitrogen present other than as ammoniacal nitrogen. Evaporate 500 ml. of sample to 250 ml to remove free ammonia. Add 5 ml. of concentrated sulfuric acid and a small piece of pumice. Mix and digest in a suitable flask until copious fumes of sulfur trioxide are given off and the liquid is colorless or very light yellow.

If necessary for complete decomposition of organic matter, add 5 grams of anhydrous sodium or potassium sulfate in order to raise the temperature of the boiling sulfuric acid. When decomposition is complete and the flask has cooled, dilute to 500 ml. and treat as for ammoniacal nitrogen. The phenol-sodium hypochlorite reagent is also satisfactory for water analysis.[5]

Sea Water. For ammonia in sea water the usual Nessler reagents have a nonsensitive region up to 0.03-0.08 mg. per liter. Beer's law does not apply below 0 02 mg. per liter with the special reagent found most satisfactory. For sea water, therefore, prepare Treadwell's form of Nessler's reagent.

Blood. *Nonprotein Nitrogen.* Pipet 1 ml. of blood or plasma into a 10-ml. volumetric flask, add 8 ml. of 1:400 sulfuric acid, and mix by swirling gently. Add 1 ml. of 10 per cent sodium tungstate solution, bringing the liquid level to the 10-ml. calibration mark. Stopper and mix by inverting 10 times. Allow to stand for 5 minutes and filter the brown solution, refiltering if the initial filtrate is not clear.

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[5] Makhlis, *Novosti Tekhniki, ser. Gorno-Rudnaya Prom.* 1936, No. 25, 2.

Transfer a 1-ml. aliquot to a Pyrex test tube calibrated at 20 ml. Add a silica chip, 0.3 ml. of a 1:1 mixture of concentrated sulfuric acid, and a 0.3 per cent solution of copper sulfate pentahydrate. Heat the tube at a slant for 2-3 minutes until charring begins. Cool for 30 seconds and add dropwise 0.4 ml. of a 2.5 per cent solution of potassium persulfate. Insert a small funnel and continue heating for 1 minute or until the digest becomes clear. Cool for 30 seconds and add 2 drops more of persulfate solution through the funnel. Heat for 1 minute to complete digestion, making certain that the heat is not sufficiently high to cause fumes to escape from the tube. Cool and dilute to the 20-ml. mark for use in the determination of nitrogen as ammonia by nesslerization, using 5 ml. of reagent. A normal content is 0.0003-0.00056 per cent.[6]

Urine. This method separates the ammonia by its base exchange reaction with Permutit. In neutral or faintly acid solution with sodium salts low, the absorption is practically quantitative. In strongly alkaline solution the ammonia is liberated. A 60-80 mesh grade of Permutit is suitable. The method should be applicable to many other liquids.

Place 2 grams of Permutit in a 200-ml. volumetric flask. Add 5 ml. of distilled water and 2 ml. of urine. Rinse the pipet with 5 ml. of water and shake gently for 5 minutes. Rinse the sides of the flask with about 50 ml. of water from a wash bottle and decant. If there is appreciable color left, rinse 2-3 times more. At this stage the ammonia is present in the Permutit. Add 5 ml. of water and 5 ml. of 10 per cent sodium hydroxide solution to displace the ammonia. Dilute to volume, let stand for 15 minutes, and pipet 50 ml. of solution as sample.

The absorbed ammonia is more difficult to remove if left in the Permutit overnight. About 95 per cent of the ammonia is liberated in 3 minutes and all in 10-15 minutes. This may vary with the Permutit. After rinsing with water, with 2 per cent acetic acid, and again with water, the Permutit may be used again and is as efficient as before.

Milk. Take a suitable sample giving 2-5 mg. of nitrogen and digest with 1-2 ml. of a mixture of 70 ml. of concentrated sulfuric acid, 50 ml. of water, 20 ml. of 20 per cent perchloric acid, 15 grams of anhydrous sodium sulfate, and 1 gram of copper sulfate. The perchloric acid is not essential. Heat for at least 2 minutes after the solutions are colorless. Cool, dilute, add 5 ml. of protective colloid prepared from gum arabic,

[6] W. Hurka, *Mikrochemie ver. Mikrochim. Acta* **33**, 11-19 (1947).

(page 816), and 2-3 drops of Nessler's reagent as indicator. Neutralize with 10 per cent sodium hydroxide solution and dilute to 100 ml. Take 50 ml. of this as sample.

Plant Material.[7] *Total Nitrogen.* Prepare a solution of sample as described under potassium (page 550). Transfer a 5-ml. aliquot to a 50-ml. volumetric flask. Add 1 ml. of a 10 per cent solution of sodium hydroxide and 1 ml. of 10 per cent sodium silicate, dilute to volume with water, and mix well. Use 5-ml. aliquots for the determination of nitrogen by Nessler's reagent.

Sewage. *Ammoniacal Nitrogen.* Direct nesslerization is preferable since distillation may give high results, especially after addition of sodium carbonate. To 100 ml. of sample add 1 ml. of a 10 per cent copper sulfate solution and mix. Add 1 ml. of 50 per cent sodium hydroxide solution, mix, and let settle. If the supernatant liquid does not become perfectly clear, repeat with a fresh sample adding the alkali first. If the sample contains hydrogen sulfide, replace the copper sulfate with lead acetate and if that is ineffective, with zinc sulfate. Dilute a suitable aliquot, which is usually 5 ml. or less, to 50 ml. and use as sample.

Alternatively, use 10-100 ml. of sample diluted to 500 ml. and treat by distillation, the same as for water samples (page 810). If acid to methyl orange, neutralize with 10 per cent sodium carbonate solution before distillation.

Albuminoid Nitrogen. Determine as in water (page 811).

Organic Nitrogen. This determination must be made promptly after sampling as loss occurs progressively in unsterilized sewage. Take 100 ml. or less of sample in a Kjeldahl flask and distill off the free ammonia. Add 10 ml. of concentrated sulfuric acid, 0.1 gram of copper sulfate, and 5 grams of sodium or potassium sulfate. Digest over a low flame until it becomes colorless and for 30 minutes longer. When cool, dilute to 250 ml. with ammonia-free water. Add a few drops of phenolphthalein solution and 50 per cent sodium hydroxide solution until alkaline. Distill as described under water (page 811). Correct the result obtained for a blank on the reagents.

Alternatively, determine by direct nesslerization This is less accurate than distillation but is preferable for routine work. Digest as for the

[7] Robert H. Cotton, *Ind. Eng. Chem., Anal. Ed.* **17**, 734-8 (1945).

distillation method. Dilute the digested liquid to 250 ml. in a calibrated flask. Pipet 50 ml. into a 100-ml. flask and neutralize with 50 per cent sodium hydroxide solution, keeping the flask cooled with water. When it shows a color to phenolphthalein dilute to volume and let stand for 24 hours. Take an aliquot of the supernatant liquid as sample for Nessler determination. Correct for a blank on the reagents.

Feed and Other Dry Materials. Digest a suitable sample by the Kjeldahl method. Either distillation or direct nesslerization may be used.

Leather.[8] Follow the conventional Kjeldahl digestion by distillation and apply the Nessler method.

Standards

Ammonium Chloride. Dissolve 0.3820 gram of ammonium chloride in water and dilute to 1 liter. This solution contains 0.1 mg. of nitrogen per ml. in the form of ammonia. If a more dilute solution is required, dilute a 10-ml. aliquot to 100 ml. The latter solution contains 0.01 mg. of ammonia nitrogen per ml.

Ammonium Sulfate. Add 10 ml. of 7.5 per cent potassium sulfate solution to 10 ml. of an ammonium sulfate solution containing 0.0929 gram per liter. Dilute to 100 ml. Each ml. is equivalent to 0.002 mg. of nitrogen as ammonia.

Nitrogen as Ammonia by Nessler's Reagent

In reaction with ammonia Nessler's reagent produces a yellow to reddish brown colloidal dispersion which is a very accurate indicator of the amount of ammonia present. This reaction is used for examination of many biological materials, as well as for specific forms of organic nitrogen, to be discussed in detail in Volume 3. The conventional method has been to compare with artificial standards or, occasionally, with natural standards. Spectrophotometric reading at 410 mμ is suitable.[9]

Nessler's Reagent. Because of the wide variety of applications of Nessler's reagent, there are numerous modifications of which several are given here.

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[8] F. K. Fisher, *Abhandl. Staatsuniv. Saratov.* **1**, 107-12 (1936).

[9] H. F. Beeghly, *Ind. Eng. Chem., Anal. Ed.* **14**, 137-40 (1942); Margherita Marzadro, *Ann. chim. applicata* **35**, 231-5 (1945).

Jackson's Modification. Dissolve 50 grams of potassium iodide in about 35 ml. of cold ammonia-free water. Slowly add a saturated solution of mercuric chloride until a slight red precipitate appears. Add 400 ml. of clear 36 per cent sodium hydroxide or 50 per cent potassium hydroxide solution. Dilute to 1 liter, let stand to sediment, and decant. This reagent, properly prepared, will give a color with 0.001 mg. of ammonia in 50 ml. of water within 5 minutes and will not produce a precipitate with a reasonable amount of ammonia within 2 hours. One ml. is normally used for a 50-ml. sample. The reagent is stable and can be used indefinitely.

Folin-Wu Modification. A more dilute reagent for biological use is as follows: Put 150 grams of potassium iodide and 110 grams of iodine in a large flask. Add 100 ml. of water and 140-150 grams of metallic mercury. Shake vigorously until the color of iodine has nearly disappeared. Cool in water and continue shaking until the reddish iodine color has been replaced by the greenish color of the double iodide. Decant from the excess mercury and wash the latter. Dilute the solution and washings to 2 liters. Put 3500 ml. of a 10 per cent solution of sodium hydroxide, made by the dilution of a saturated solution, into a 5-liter flask. Add 750 ml. of the stock double-iodide solution, and 750 ml. of distilled water. Use 15 ml. of this in a total of 50 ml. containing the sample.

Treadwell Modification. A special form of Nessler's reagent found best for sea water is prepared as follows: Dissolve 115 grams of mercuric iodide and 80 grams of potassium iodide in enough water to make 500 ml. Add 500 ml. of 24 per cent sodium hydroxide solution.

Procedure. If distillation is to be carried out, pipet an aliquot containing 0.02-0.2 mg. of ammonia into a 100-ml. Kjeldahl flask. If carbonates are present, just neutralize with 1:50 sulfuric acid. Add a chip of granite and dilute to 30 ml. Immerse the tip of the delivery tube in 23 ml. of freshly prepared 0.0013 N sulfuric acid. Connect with the air condenser, mix the contents of the flask by swirling, and heat to distill 15 ml. Disconnect the delivery tube and rinse into the dilute sulfuric acid solution. Transfer the solution quantitatively to a 100-ml. flask and dilute to volume.

Unbuffered Development.[10] After distillation or, if distillation is

[10] R. F. Reitemeier, *Ind. Eng. Chem., Anal. Ed.* **15**, 393-402 (1943).

unnecessary, add a suitable amount of Nessler's reagent, Jackson's modification, depending on the amount of sample. Usually 1 ml. suffices. Mix and, after 15 minutes, compare with standards or read the transmittance at 420 mμ, using a reagent blank as reference. The color is stable for about 30 minutes. A blue filter facilitates the reading.

Buffered Development. Transfer the sample to a 100-ml. volumetric flask. Take an equivalent standard in a similar flask and dilute to about the same volume. Add 1 ml. of 0.01 N sodium hydroxide solution saturated with thymolphthalein to each. Neutralize the solutions with 2 per cent sodium hydroxide solution. Prepare a borate buffer by dissolving 12.404 grams of boric acid in 100 ml. of carbonate-free 4 per cent sodium hydroxide solution and diluting to 1 liter with water. Add 20 ml. of this buffer to sample and standard.

Protective colloids such as gelatin or gum arabic solution may be advantageously added at this point. To prepare the latter, add 10 grams of powdered gum arabic slowly with vigorous stirring to 190 ml. of water. Stir until the gum is completely dispersed. Transfer to a flask and add 4 grams of Permutit powder. Shake at intervals for 10 minutes and let stand to settle. After a few minutes decant the slightly turbid supernatant liquid. Test a portion to see that it gives only a faint coloration with Nessler's solution. If it gives a distinct reaction, repeat the treatment with Permutit powder. To the colloid add about one-tenth its volume of Folin and Wu's Nessler solution to remove reducing materials, allow to stand and decant when ready for use. By use of 2.5 ml. of this solution per 50 ml. of final solution, as high as 10 mg. of nitrogen may be estimated in 50 ml. Satisfactory results have been obtained on solutions saturated with sodium sulfate.

Dilute the flasks of sample and standard to about 97 ml. and add 2 ml. of Nessler's reagent. Dilute to volume and mix. Compare after 1 hour but not more than 2 hours. The final pH is about 12.0. The slight blue due to the indicator is the same in sample and standard.

Artificial Standards. *Platinum-cobalt.*[11] Prepare a solution of 2.0 grams of potassium chloroplatinate in 100 ml. of concentrated hydrochloric acid and a solution of 12 grams of anhydrous cobaltous chloride in 100 ml. of similar acid. Dilute each to 1 liter.

As standards mix the volumes indicated in Table 13 and dilute each to 50 ml. in standard Nessler tubes. Compare with natural standards and

[11] Standards Methods for the Examination of Water and Sewage, (Ninth Edition), p. 67. American Public Health Association, New York, N. Y. (1946).

adjust as necessary to match the natural standards within the limit of accuracy of observation.

TABLE 13. PERMANENT PLATINUM-COBALT STANDARDS FOR AMMONIA NITROGEN BY NESSLER'S REAGENT

Value in Ammonia Nitrogen (mg.)	*Volume of Platinum Solution (ml.)*	*Volume of Cobalt Solution (ml.)*
0.000	1.2	0.0
0.001	1.8	0.0
0.002	2.8	0.0
0.004	4.7	0.1
0.007	5.9	0.2
0.010	7.7	0.5
0.014	9.9	1.1
0.017	11.4	1.7
0.020	12.7	2.2
0.025	15.0	3.3
0.030	17.3	4.5
0.035	19.0	5.7
0.040	19.7	7.1
0.045	19.9	8.7
0.050	20.0	10.4
0.060	20.0	15.0
0.070	20.0	22.0

Chromate-cobalt. Prepare an aqueous solution containing 0.8 ml. of 10 per cent potassium chromate solution and 22 ml. of 10 per cent hydrated cobalt nitrate. This corresponds to 0.01 gram of ammonia per liter.

AMMONIA NEPHELOMETRICALLY BY A MODIFIED NESSLER'S REAGENT

Nessler's reagent tends to give a cloud if the concentration of ammonia is relatively high. The color developed is also affected by dissolved salts. A modified form of Nessler's reagent has therefore been developed for nephelometric use. This reagent is sodium mercuric chloride instead of the potassium mercuric iodide used as Nessler's reagent. This is more stable and the complex does not have the appreciable ammonia vapor tension of the corresponding ammonia-potassium mercuric iodide complex.

Salts do not affect the formation of the colloidal precipitate. As a matter of good practice the amount in the sample is preferably duplicated in the standard. The method will detect 1 part of ammonia in 160 million parts of solution. If the sample is prepared by Kjeldahl digestion without distillation, copper must not be used because of the color it would impart to the final solution. The most satisfactory method of reduction of nitrates is with titanous chloride.[12] The reduction of nitrites is not complete.

Procedure. To prepare the reagent, add 80 grams of sodium chloride to 130 ml. of water. Add 100 ml. of a cold saturated solution of mercuric chloride and shake. When solution of the salt is complete, add 70 ml. of a 1 per cent solution of lithium carbonate. This is practically a saturated solution. Make this addition slowly with shaking so that no mercuric oxide forms on the sides of the flask. Some cloud is usually present due to ammonia in the reagents.

Shake the reagent with 3-5 grams of finely powdered talc and filter. It may be used at once and will keep indefinitely if properly protected.

To one comparison tube add a 10-ml. aliquot of sample. To another add 10 ml. of ammonium sulfate standard containing excess of potassium sulfate. To each add 15 ml. of a 0.003 per cent starch solution prepared by boiling 1 gram in 100 ml. of ammonia-free water and diluting 3 ml. to 1 liter. To each add 5 ml. of reagent. Compare nephelometrically.

Ammonia by the Phenol-Sodium Hypochlorite Reagent

In alkaline solution ammonia forms an intense blue dye with a phenol-sodium hypochlorite reagent which is proportional to the amount of ammonia present.[13] Ordinarily, in strongly alkaline solution, the reaction at 37° takes about 1-2 hours for development and is not as intense as that produced at 100°. The latter is not readily reproducible. By maintaining the pH at 12, so that it is equivalent to the molar strength of the phenol present, and conducting the reaction at 100°, a color forms that is 3 times as intense as that with the more alkaline reagent.

Iron, chromium, and manganous ions act as catalysts for the development of color and increase its reproducibility, whereas copper retards

[12] Benjamin Wolf, *Anal. Chem.* **19**, 334-5 (1947).

[13] Donald D. Van Slyke and Alma Hiller, *J. Biol. Chem.* 102, 499-504 (1933); Jane A. Russell, *J. Biol. Chem.* **156**, 457-61 (1944); A. V. Yanush and A. E. Voitse-Khovskii, *Khim. Prom.* **1946**, No. 7/8, 14-15.

the development of color. The presence of free acid also interferes. The color is stable for 1 hour. It is possible to detect 0.1 ppm. of ammonia nitrogen per ml. The solution has a minimum transmittance at 625 mμ.

Procedure. Use a 5-ml. sample in neutral or 0.01 N acid solution containing 0.002-0.02 mg. of ammonia nitrogen. Transfer to a calibrated 10-ml. tube and add 1 drop of a 0.5 per cent solution of manganese chloride.

To prepare an alkaline phenol reagent, mix 25 grams of crystalline phenol with water and stir in 54 ml. of a 20 per cent solution of sodium hydroxide. Dilute to 100 ml. and keep refrigerated in a dark bottle. To prepare the accompanying hypochlorite reagent, dissolve 25 grams of finely ground calcium hypochlorite in 300 ml. of hot water. Add 135 ml. of a solution of 20 grams of anhydrous potassium carbonate which has been boiled in 100 ml. of water to remove ammonia. Mix and heat just to 90°. Cool and dilute to 500 ml. Filter a small portion of the mixture and to 1 ml. of filtrate add 3 ml. of the potassium carbonate solution. Heat in a boiling water bath for 5 minutes. A solution that remains clear indicates the absence of calcium. If the reaction is positive, treat with additional potassium carbonate solution until the reaction for calcium is negative. Filter and refrigerate in brown bottles. About 0.013-0.014 gram of free chlorine per ml. is present.

To the sample tube in an ice bath add 1 ml. of alkaline phenol reagent and 0.5 ml. of hypochlorite solution. Mix by rotation and place in a boiling water bath for 5 minutes. Cool, dilute to volume, and compare with suitable standards. Alternatively, read the transmittance and apply a calibration curve.

Miscellaneous

A fresh mixture of silver nitrate and tannin gives a color due to formation of free silver which is more sensitive than Nessler's reagent.[14] To 1 ml. of sample add 2 drops of 5 per cent tannin solution and 1 drop of 20 per cent silver nitrate solution. A color appears at once and is compared with a series of standards after a minute.

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[14] Konstantin C. Makris, *Z. anal Chem.* **81**, 212-14 (1930); *ibid.* 84, 241-2 (1931).

standard so that each ml. represents 0.01 per cent of carbon. If the standard were 0.54 per cent of carbon, it would then be diluted to 54 ml.

Series-of-standards Method. Weigh into tubes one or more samples of 0.5 to 1.0 gram and a sufficient number of standards of similar weight to cover the desired range of expected carbon contents. To each add 10 to 20 ml. of 1:1 nitric acid and heat with occasional shaking on a hot plate or in a boiling water bath. When all are dissolved, remove, cool, and dilute with water to a standard volume which is not less than double the volume of acid used. Estimate the carbon content of the samples from the series of standards.

Combined Carbon in Iron. Transfer 1-gram portions of sample and a similar standard of iron to beakers, add 30 ml. of 1:1 nitric acid to each and heat until decomposed. Filter off graphitic carbon and silica, washing the filters with water until the filtrate comes through colorless. Collect the filtrates in 100-ml. volumetric flasks, dilute to volume, and mix. Transfer suitable volumes to comparison tubes and compare the color, diluting standard or sample with water as necessary.

Carbon in Organic Matter by Conversion to Carbon Dioxide

Carbon present in organic samples may be estimated by oxidizing it to carbon dioxide and collecting the evolved gases.[2] Sodium chlorate and 50 per cent sulfuric acid are used as the oxidizing agents by a modification of a procedure for determining nitrogen. The carbon dioxide is estimated from its effect on the sodium salt of phenolphthalein. Full details of preparation of the sample have been given under nitrates (page 789).

Procedure. Connect C to the apparatus shown in Figure 35. The tube to D is now above the liquid in C. Put a small volume of water in G. Fill flask H and cylinder F with water. Open B and D and start a siphon by applying suction at I. Close D. Remove the water from G and replace with the proper volume of solution of the sodium salt of phenolphthalein indicated by the method on page 848. Lower the level of the water in F to that of the water in C. Open D to equalize the pressure in C. Measure the exact volume of water in F with the tube at the water level. Raise F and gas will be forced from C into G and H. Water will flow

[2] E. M. Emmert, *J. Assoc. Official Agr. Chem.* **12**, 240-7 (1929); *ibid.* **13**, 146-8 (1930); *ibid.* **14**, 386-9 (1931); *ibid.* **16**, 424-7 (1933).

from F into C and from H into J. After 70-80 ml. of gas have passed over, again lower F and read the water level as before. The loss in volume is due to the gas which has passed into G. Close D and shake G intermittently but vigorously for 5-10 minutes, at which time no further reduction in color of G should occur. By the use of a proper amount of tubing this can be done without disturbing C or H.

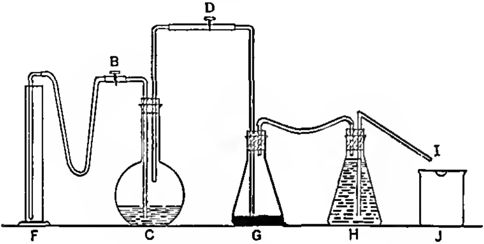

Fig. 35

Apparatus for Estimation of Carbon Dioxide in Mixed Gases

Compare the color of the sodium salt of phenolphthalein, by the indicator method (page 843), in order to estimate the total amount of carbon dioxide in the volume of gas passed into G. Divide this amount of carbon dioxide in mg. by the volume in ml. of gas used, to give mg. of carbon dioxide per ml. of gas. Repeat the determination of carbon dioxide in the gas until checking results are obtained. Results on the first two aliquots are likely to be low. When a duplicable value has been obtained, multiply by the volume of gas in C after oxidation and calculate to carbon in the original sample.

CHAPTER 63

CARBON MONOXIDE

CARBON MONOXIDE assumes a position of great importance, so far as determination in gases is concerned, because of its toxicity and its odorless nature. The sources are legion—the vicinity of a leaking flue, the proximity of an automobile exhaust, or some areas in plant operation where carbon monoxide is evolved. Up to 100 ppm. can be tolerated but it is essential that quantitative methods be available at much below that level. Detection of the gas already absorbed by the blood is important in diagnosis of carbon-monoxide poisoning.

The methods most widely used apply the palladous chloride reagent, but iodine pentoxide is important, as are the older tannic reagents and blood itself. Because of the very specific form of application of the methods, the arrangement within a chapter as described in the Foreword will not be followed in this chapter.

CARBON MONOXIDE BY PALLADIUM CHLORIDE

When carbon monoxide is brought into contact with palladium chloride solution, reduction to metallic palladium occurs according to the reaction $CO + PdCl_2 + H_2O \rightarrow Pd + CO_2 + 2HCl$. This reaction is applied to detection [1] of carbon monoxide and to its estimation in large amounts [2] or in lesser concentrations. Variations of the method consist of drawing the gas through paper impregnated with the reagent, suspending impregnated paper in the gas with or without agitation or heat,[3] exposing impregnated silica gel granules,[4] and exposing the solution to the gas. The time of contact would restrict the degree of reaction in all of these cases.

While acetone accelerates the reaction,[5] other solvents for carbon monoxide do not, and a lower temperature, which would increase the

[1] R. Böttger, *J. prakt. Chem.* 76, 233-5 (1859).
[2] C. Winkler, *Z. anal. Chem.* 28, 269-78 (1889).
[3] A. Lambrechts and R. Roseman, *Compt. rend. soc. biol.* 140, 801-3 (1946).
[4] John D. Main-Smith and George A. Earwicker, British Patent 582,184 (1946).
[5] Chemical Department of the South Metropolitan Gas Co., *J. Soc. Chem. Ind.* 57, 79-82T (1938).

solubility, slows down the reaction. Approximately 90 per cent of the carbon monoxide reacts in the first 2 hours of exposure to a static solution, but 10 hours at 40° are required to reduce the unreacted amount to a negligible value. Small errors of time or temperature have little effect at 2 hours. Shaking for 2 hours practically completes the reaction at room temperature. A saturated solution of chromic acid in sulfuric acid will efficiently wash out unsaturated hydrocarbons, aldehydes, etc., which would otherwise react. Oxides of nitrogen need not be removed. Free hydrochloric acid is necessary, but more than 0.001 *N* retards the reaction. For up to 120 ppm., test paper is satisfactory; above that to 500 ppm. one must dilute four-fold due to the limited amount of reagent on the test paper. By shaking with reagent the limit goes up to 4000 ppm.

As applied in the form of a simple device[6] for testing the air of sewers and confined spaces, when the temperature of the air is above 10° the results are semi-quantitative in 10 minutes at 200-1000 ppm. At 0°, 20-30 minutes are required, and at −16° a satisfactory result was not obtained in 30 minutes. Gasoline vapor, ethylene, hydrogen, and hydrogen sulfide also give the test, but the concentrations which react are also dangerous.

In a modified form,[7] which will not be presented in detail, three tubes with inlets leading to the bottom are connected in series. The first contains a 1 per cent solution of palladium chloride in 1:100 hydrochloric acid to remove any reactive material from the incoming air. The second or sample tube contains 2 ml. of blood. 4 ml. of an aqueous solution of 3.2 grams of potassium ferricyanide and 0.8 gram of saponin per 100 ml., and 2 drops of caprylic alcohol. The third tube contains glass beads and 5 ml. of 10 per cent lead acetate solution to remove sulfides. These tubes are followed by a disc of filter paper wet with the same solution as in the first tube and so held between flanges that the air must pass through it. Lastly an aspirator bottle draws air through the system. The stain on the paper is compared with standards similarly prepared with blood of known carbon monoxide content.

In a more detailed method[8] instead of determining the precipitated

[6] L. B. Berger and W. P. Yant, *U. S. Bureau of Mines Report of Investigation* 3030; Chester S. Gordon and James T. Lowe, U. S. Patent 1,644,014 (1927); L. B. Berger and H. H. Schrenk, *U. S. Bureau of Mines Tech. Paper* 582 (1938); sold by Davis Emergency Equipment Co., Inc., 67 Wall St., New York, N. Y., and by Mines Safety Appliances Co., Pittsburgh, Pa.

[7] Alexander O. Gettler and Henry C. Freimuth, *Am J. Clin. Path., Tech. Sect.* **7**, 79 82 (1943).

[8] Adam A. Christman, Walter D. Block and Julius Schultz, *Ind. Eng. Chem., Anal. Ed.* **9**, 153 6 (1937).

palladium gravimetrically, volumetrically, or by eye, the excess palladium chloride is converted to palladous iodide by dissolving in excess potassium iodide, and the red color is read. Analyses of samples containing 100-800 ppm. of carbon monoxide show recoveries averaging 95 per cent and are 94-99 per cent of those by the iodine pentoxide method.[9]

The time for initial appearance of metallic palladium is a rough measure of the carbon monoxide present, thus somewhat paralleling the detector method, even though acetone is not used to activate. For 1000, 800, 600, 400, and 200 ppm. at 20-25° C. it is 5, 12, 17, 35, and 60 minutes within an accuracy of about 5 minutes. Concentrations above 1000 ppm., which are dangerous to breathe for even a brief time, give an almost immediate black scum. Amounts of hydrogen up to 0.4 per cent by volume did not interfere within 24 hours. Ethylene at 600-800 ppm. showed reduction in 3-4 hours. Provision for removal of reducing gases is made in sampling.

Detector. The detector is a thin-walled glass tube about 5 × 37 mm. In this is sealed a water and acetone solution of palladium chloride. The ampoule is covered with a cotton covering and appears much like an ampoule of aromatic spirits of ammonia or of amyl nitrite. For use, crush the ampoule, which will wet the cotton covering. Lower into the space to be tested, either by a cord or by tying to a stick. If a workman is properly protected by a mask, he may carry the ampoule into the area to be tested. Expose for not less than 10 minutes.

Carbon monoxide, if present, will reduce the original brownish yellow palladium chloride to finely divided palladium and will give a yellowish black to black stain, indicating the concentration of carbon monoxide. Compare with color standards. Staining will be somewhat uneven and an area representative of the average staining is to be selected for comparison. It is desirable to expose somewhat longer than the specified 10 minutes as a safety precaution. Paper wet with the solution is an alternative.

The manufacturers furnish color charts showing the degree of coloration indicating 100-1000 ppm. of carbon monoxide. The crushed ampoule may be worn on the clothing or equipment to give warning if the carbon monoxide concentration becomes dangerously high. Precautions in terms of ppm. are as follows: Less than 100, safe: 100-600, caution, working

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[9] A. B. Lamb, W. C. Bray and W. J. Geldard, *J. Am. Chem. Soc.* 42, 1636-48 (1920); A. A. Christman and E. L. Randall, *J. Biol. Chem.* 102, 595-609 (1933); C. H. Gray and Marjorie Sandiford, *Analyst* 71, 107-10 (1946).

period not over 45 minutes with helper at hand: 600-800, extreme caution, working period not over 15 minutes with helper at hand; 800-1000, dangerous to enter.

Palladium Chloride Solution. Dry the C.P. reagent, $PdCl_2$, for 1 hour at 100° C. and weigh 0.5 gram of the dried salt into a beaker. Add 150 ml. of distilled water and 2.5 ml. of concentrated hydrochloric acid and heat until solution is complete. After cooling, transfer to a 500-ml. volumetric flask and dilute to volume.

Sample. Figure 36 shows the apparatus for sampling. The volume of the 500-ml. flask A up to the 2-way stopcock B must be determined and will usually be 528-532 ml. For simplicity of later calculation it is desirable to adjust the degree to which the stopcock protrudes through the stopper so as to approximate this volume. The reservoir C has a capacity of about 7 ml. The stopcock should protrude as a minimum about 1 cm. through the one-hole rubber stopper to avoid contact of reagent with the stopper. Several such flasks are needed.

Evacuate the flasks to less than 1 mm. of pressure and, to be sure that they do not leak, leave connected to a manometer for 24 hours. If equipment for this degree of evacuation is not available, the flasks may be evacuated to 20-25 mm. with a water pump and a suitable correction factor applied to the results. The increased oxygen pressure present is not sufficient to alter the results significantly.

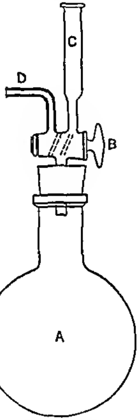

FIG. 36

Apparatus for Carbon Monoxide by Palladium Chloride

Open the stopcock of such an evacuated flask in the atmosphere to be sampled and, when equilibrium with the air has been attained, close the cock. Then to provide the sample at 0.5 atmosphere pressure connect the tube D of the flask with the tube D of another evacuated flask and open the stopcocks. Thus two samples are obtained on closing the cocks and disconnecting. If later in analysis of one of these samples the carbon monoxide content is found to be too high, connect the other sample flask at 0.5 atmosphere with an evacuated flask, thus giving half the

concentration. This may be repeated if necessary. Results so obtained must be multiplied by a suitable factor.

If hydrogen sulfide, unsaturated hydrocarbons or other interfering substances are present, wash the sample before admission to the bulb. For oxidation, dilute saturated bromine-water to one-third strength and add 5 grams of potassium bromide per 100 ml. To remove bromine vapors follow this by a wash with 33 per cent sodium hydroxide solution. The permissible rate of passage of the gas through these wash solutions will depend on the efficiency of contact. Practically, in control work on analysis of garage air, such washing is unnecessary.

Procedure. Put 3 ml. of the palladium chloride solution, carefully measured, into the reservoir **C** and add 0.2 ml. of a 10 per cent aluminum sulfate solution. Transfer this to the flask quantitatively by carefully opening the stopcock, and wash in with three 1-ml. portions of distilled water. No air must enter the flask during this operation The presence of oxygen in the flasks tends to cause low results. Where the determinations are carried on at 0.5 atmosphere, if carbon monoxide-free air is admitted to the flask to raise the pressure to 1 atmosphere, results will be 5-10 per cent lower.

Shake the flask at intervals for the next 2 hours particularly during the early part of the reduction in order to avoid forming a layer of palladium on the surface of the reagent. The aluminum sulfate is present to flocculate the palladium formed. Let stand for at least 4 hours and preferably overnight before proceeding to the next step. Results at the end of 4 hours are slightly lower on the average than after longer standing.

Release the pressure in the flask by admission of carbon monoxide-free air and transfer the contents of the flask to a filter which has a 50-ml. volumetric flask as receiver. Wash the flask and paper well, so that there are about 25-30 ml. of clear solution in the receiver. Add 2 ml. of recently filtered 1 per cent gum ghatti solution and mix, then 5 ml. of 15 per cent potassium iodide solution and mix again. To remove traces of palladous chloride sorbed on the filter paper, wash the filter with a 2-ml. portion of the potassium iodide solution, then with a few ml. of water. Repeat this operation with another 2-ml. portion, then with 1 ml. Be sure to wash thoroughly after the last so that a total of 10 ml. of potassium iodide solution will have been added. Add a drop or two of caprylic alcohol to the flask to minimize foaming, dilute to volume and mix. The color is fully developed in a few minutes and is constant for at least 24 hours.

Compare with the developed standard by balancing and calculate the results to palladium chloride reduced by the sample. One mg. of palladium chloride is reduced by 0.1261 ml. of carbon monoxide at 0° C. and 760 mm. If the volume of sample was 530 ml. at 0.5 atmosphere, the calculation can be simplified to 1325 × milligrams of palladium chloride reduced, × absolute temperature at which sample was taken / barometric pressure in mm. at which sample was taken = ppm. of carbon monoxide.

The recovery of palladous chloride from metallic palladium is not complete and becomes greater with increase in the metallic palladium formed. To correct for this, subtract from the final result predetermined corrections. For 0.5, 1.0, 1.5 and 2.0 mg. of palladium chloride reduced, these are 19, 28, 33, and 38 ppm. of carbon monoxide.

Standard. Mix 2 ml. of the palladium chloride solution, 25 ml. of water, and 2 ml. of the gum ghatti solution in a 50-ml. volumetric flask. Add 10 ml. of 15 per cent potassium iodide solution, mix, dilute to volume, and mix.

Carbon Monoxide as Iodine

When carbon monoxide is oxidized by iodine pentoxide, the products are carbon dioxide and iodine.[10] Instead of determining the carbon dioxide or the iodine titrimetrically, the latter may be estimated colorimetrically.[11] This permit a check determination on the same sample and avoids the inconveniences associated with titration of solutions of low normality. Below 50 ppm. or 0.005 per cent of carbon monoxide the colorimetric method is definitely preferable. In the range below 100 ppm. or 0.010 per cent, accuracy to 5-10 per cent is obtained. Other methods for determination of small amounts of iodine (Chapter 53) are applicable. Interfering gases, except hydrogen, are removable. Unless hydrogen is in excess of the carbon monoxide, it may be neglected. For small concentrations, 1600-1700 ml. samples are preferable, although 200-250 ml. samples can be used. The heated iodine pentoxide at the temperatures used gives an appreciable blank because of dissociation. Therefore, the vapor space must be freshly flushed out and the amount of gas used for purging must be standardized.

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[10] M. C. Teague, *Ind. Eng. Chem.* **12**, 964-8 (1920); American Gas Association, *Gas Chemists' Handbook*, 3rd Ed., pp. 289-95. New York, N. Y. (1929); J. S. Haldane and J. I. Graham, *Methods of Air Analysis*, pp. 116-29, London (1935); L. B. Berger and H. H. Schrenk, *U. S. Bur. of Mines, Tech. Paper* 582, 1-30 (1938).

[11] Bernard Smaller and John F. Hall, Jr., *Ind. Eng. Chem., Anal. Ed.* **16**, 64-6 (1944).

Procedure. The apparatus for oxidation is shown in Figure 37. Attach the sampling hulb containing the gas in a vessel of water to a float-operated valve. Apply suction at the exit of the ahsorption hulh to draw the gas sample through the liquid air scruhher, the iodine pentoxide tube heated to ahout 150° in an oil bath, through the trap to the absorher. Regulate the rate at which the gas is passed hy the pinch clamp on the

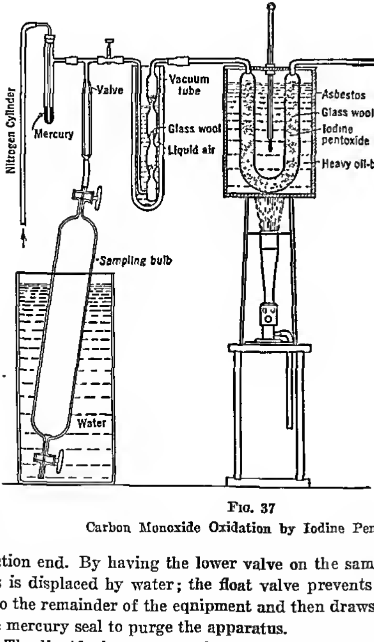

Fig. 37
Carbon Monoxide Oxidation by Iodine Pentoxide

suction end. By having the lower valve on the sampling hulh open, the gas is displaced hy water; the float valve prevents water from passing into the remainder of the eqnipment and then draws in nitrogen through the mercury seal to purge the apparatus.

The liquid air condenser is nsed for automohile exhaust gases. An alternative for combustion prodncts is a solution of chromic acid in concentrated sulfuric acid heated to 100° to remove sulfur dioxide, hydrogen

sulfide, and unsaturated hydrocarbons. It should be followed by a tube of phosphorus pentoxide to remove moisture and acid spray.

Various other absorbers have been used. One for mine atmospheres is a series consisting of bromine in potassium bromide-sodium hydroxide solution-solid sodium hydroxide-activated carbon-phosphorus pentoxide. Another [12] for ethylene is the series fuming sulfuric acid-concentrated sulfuric acid-soda lime-phosphorus pentoxide.

The phosphorus pentoxide must be of maximum purity. Before use, condition it by heating at 220-250° while passing nitrogen over it free from carbon monoxide, until a small, constant blank is obtained. A charge of 30-40 grams arranged in layers on glass wool is satisfactory. This will care for 50-100 ml. of gas per minute. More than 0.3 per cent of carbon monoxide may result in condensation of iodine on glass surfaces, requiring more than usual purging. Therefore, joints between the oxidation tube and the absorption bulb must be of glass.

Pass a 1600-1700 ml. sample in 60 minutes followed by 30 minutes' purging. Absorb the liberated iodine in 10 ml. of 1 per cent potassium iodide solution. Dilute to such extent as necessary with 1 per cent potassium iodide solution and read within 10 minutes after absorption is completed.

Because of the difficulty of producing natural standards by the reaction and the necessity for prompt comparison, such standards are desirably prepared by adding known amounts of iodine standard to potassium iodide solution. By determination of transmittance the whole problem of standards is much simplified. Maximum absorption occurs in the ultraviolet, but at 350 mμ the absorption is over 90 per cent. This is reached somewhat more conveniently by the true spectrophotometer than by the filter photometer. The duplication method is applicable, titrating into a 10-ml. portion of 1 per cent potassium iodide solution. The solution conforms to Beer's law over the range involved.

Standards. Prepare 0.001 *N* iodine solution by dilution of an accurately standardized solution of higher normality. Each ml. of this solution is equivalent to 0.056 ml. of carbon monoxide under standard conditions of temperature and pressure.

Carbon Monoxide by Hoolamite

An absorbent for carbon monoxide, called Hoolamite,[13] is prepared

[12] Wright M. Welton and N. L. Drake, *Ind. Eng. Chem., Anal. Ed.* 1, 20-24 (1929).
[13] A. B. Lamb and C. R. Hoover, *U. S. Patents* 1,321,061-2 (1919); L. B. Berger and H. H. Schrenk, *U. S. Bur. of Mines, Tech. Paper* 582, 1-30 (1938).

by mixing fuming sulfuric acid with iodine pentoxide and an inert supporting material such as pumice in such proportions that carbon monoxide reacts with the mixture to give a graded series of colors. It is applicable in the range 1000-10,000 ppm. or, by modification, at 500-2000 ppm. These are 0.10-1.0 per cent and 0.05-0.2 per cent respectively.

The white granules change to increasing depths of bluish green, then to violet and finally to black. As would be expected, moisture causes deterioration. Gases such as acetylene, alcohol, ammonia, benzene, ether, ethylene, gasoline, hydrogen sulfide, arsine, hydrocyanic acid, hydrogen chloride, and natural gas containing some of the higher paraffin hydrocarbons produce a similar effect but may be removed by passing the sample of mixed gases through activated carbon. Hydrogen, methane, sulfur dioxide, carbon dioxide, carbon tetrachloride, chlorine, nitrogen pentoxide, and phosgene are without action. The green color disappears in a few minutes, so that the same reagent can be used several times.

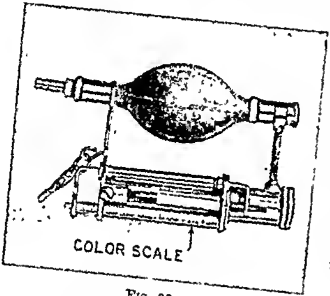

FIG 38
Carbon Monoxide Detector.
(Mines Safety Appliances Co.)

Sample. *Manholes and Confined Spaces.* Without the ampoule in place, connect a rubber tube of about 3 mm. diameter to the tip of an instrument such as that shown in Figure 38[14] and let the tubing hang down into the space to be sampled. Squeeze the bulb at least once for every foot of 3 mm. tubing and correspondingly more for large tubing, in order to draw the sample to the instrument. The instrument is shown as a diagram in Figure 39.

Reagent. Mix 11 parts by weight of iodine pentoxide with 55 parts by weight of fuming sulfuric acid containing 80 per cent sulfur trioxide and add 34 parts by weight of granular pumice. When properly protected in closed containers, the reagent increases in activity for several days and then remains unchanged, apparently indefinitely. Prepare

[14] Mines Safety Appliances Co., Pittsburgh, Pa.

tubes of reagent 5 mm. in diameter and 50-70 mm. long. The commercial instrument is provided with reagent ampoules. Before use of the commercial ampoules, break off both tips at marks previously scored on them and insert the ampoule in the instrument.

Procedure. Pass 500 ml. of the gas sample through activated carbon if necessary and then through the detector in 30-60 seconds. Compare

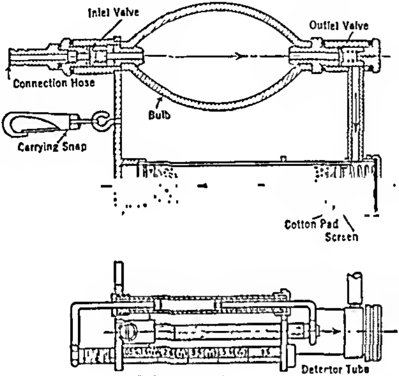

FIG. 39

Diagram of the Carbon Monoxide Detector

at once with the standards described to estimate the percentage of carbon monoxide present.

Commercial Detector. For use of the commercial detector, prior to insertion of the ampoule, squeeze the bulb of the instrument several times to make sure that it is filled with the same gas as that to be tested. After insertion of the ampoule squeeze the bulb, collapsing it completely, and allow it to refill 10 times in succession. This forces about 700 ml. of gas through the tube. Some sulfur trioxide should be emitted from the tube when the bulb is squeezed. Compare at once with the color scale on the instrument. If no color appears after 10 squeezes, the car-

bon monoxide content is less than 1000 ppm., or 0.1 per cent. Squeeze the bulb 10 times more. If no color appears in 20 squeezes, the carbon monoxide content is under 500 ppm., or 0.05 per cent. If color appears in 1-2 squeezes, the content of carbon monoxide is high enough to be dangerous to the person making the test, unless protected by a mask. The results are additive, hence if 20 squeezes are used, divide the recorded value by 2. Similarly, if only 5 squeezes give a color on the scale, multiply the result so read by 2.

Standards. Prepare permanent standards in tubes of the same size as used for the test, consisting of granules of pumice, normal or basic copper acetate and chromium oxide which match the colors produced by 500 ml. of gas containing 300-2000 ppm., which is 0.03 to 0.2 per cent of carbon monoxide according to the procedure outlined. An alternative method is to prepare a scale reading in direct percentage, to be attached to the tube in which the test is made. The commercial instrument has a calibrated scale of standards attached.

Care of Instrument. If the color fades from a tube, it can be reused. Exhaustion is indicated by a permanent yellow or green color. This is is usually after 8-10 normal tests After using, remove the ampoule and place a rubber cap over each end to keep it from contact with air until ready to use again. Leaving it uncapped for 1-2 hours will not cause serious deterioration. The commercial instrument contains activated carbon to remove gases other than carbon monoxide which would react with the reagent. This must be replaced at suitable intervals, which cannot be specified because of the variability of exposure.

Carbon Monoxide by Hemoglobin and Pyrogallic and Tannic Acids

Normal blood diluted with water and treated with tannic acid forms a gray suspension. Blood in which the hemoglobin is combined with carbon monoxide remains carmine under these conditions. This is used to determine carbon monoxide in both blood and air.[15] It is most satisfactory to use a series of permanent standards representing saturation with carbon monoxide in steps of 10 per cent. Sulfur dioxide and hydrogen sulfide must be absent from gases but are readily removed by soda lime.

If a blood sample is not over 30 per cent saturated with carbon

[15] R. R. Sayers and W. P. Yant, *U. S. Bur. of Mines, Repts. Investigations* 2356 (1922); L. B. Berger and H. H. Schrenk, *ibid.* 582 (1938).

monoxide, most of the carbon monoxide is eliminated within 8 hours.[16] Venous blood should be aerated before examination, to be sure that hemoglobin not combined with carbon monoxide is combined with oxygen. Abridged spectrophotometry is applicable.

The method gives the carbon monoxide in blood with an accuracy of 5 per cent except at very low concentrations. Accuracy to 1 per cent is obtainable[17] if (1) the standard is prepared with blood of the same species, (2) the hemoglobin content of sample and standards is identical, (3) the pH of sample and standards is identical, (4) exactly the same concentration of pyrogallol and tannic acid is used in each, (5) the sample blood is diluted 1:20, and (6) the standards are prepared in 5 per cent steps.

As applied to gas samples, the method is accurate to 0.01 per cent below 0 05 per cent, to 0.02 per cent in the range 0.05-0.10 per cent, and to 0.03 per cent in the range 0.10-0.20 per cent. In modified forms a mixture of tannic acid and hydrogen peroxide,[18] or a small amount of sodium hyposulfite,[19] $Na_2S_2O_4$, is added. In a related method the reaction of carbon monoxide in blood to reduce silver oxide in pyridine and ammonia solution at 70° has been applied colorimetrically.[20]

Procedure. *Blood.* Measure into a test tube of the same size as those used for the standard solutions, 1 ml. of 0 05 per cent potassium citrate or 0.03 per cent sodium fluoride solution, corresponding to the anticoagulant used in the standard solutions. Draw off 0.1 ml. of blood from the finger of the subject with a capillary pipet and discharge into the test tube. Add 1 ml. of a mixture of equal volumes of 2 per cent pyrogallic acid and 2 per cent tannic acid solutions or add 1 ml. of distilled water and 0.04 gram of a mixture of equal parts of tannic and pyrogallic acids. Mix with minimum agitation and let stand for 25-30 minutes at not under 19°. Compare with prepared standards. At the same time conduct a blank experiment on an unexposed subject. Alternatively,[21] dilute 0.12 ml. of blood to 10 ml. with a solution containing 3 per cent of sodium citrate dihydrate, 5 per cent of sodium bisulfate dihydrate, and 3 per cent of saponin. Read at 430 mμ and 530 mμ.

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[16] J. May, *Zeiss-Nachr.* **2**, 385-9 (1939); *Arch. Gewerbepath. Gewerbehyg.* **10**, 97 (1940); *Zentr. Gewerbehyg. Unfallverhut.* **27**, 227 (1940); *Zeiss-Nachr.* **4**, 138 9 (1942).

[17] Fumio Komatu, *J. Oriental Med.* **30**, 775-83 (1939).

[18] I. S. Ol'Kenitskiĕ, *Lab. Prakt.* (U.S.S.R.) **15**, No. 10, 25 6 (1940).

[19] H. Oettel, *Klin. Wochschr.* **17**, 1019 (1938).

[20] C. Scholten, *Deut. Z. ges. gerichtl. Med.* **30**, 292-6 (1939).

[21] Karl G. Paul and Hugo Theorell, *Acta Physiol. Scand.* **4**, 285-92 (1942).

Air or Other Gas. Dilute 0.1 ml. of normal blood to 2 ml. with addition of anticoagulant and discharge this 1:20 dilution into the sample bottle. No significant loss of carbon monoxide is caused by opening the bottle momentarily. Rotate the stoppered bottle for 15-20 minutes, avoiding shaking. Pour the blood into a small test tube, conveniently about 3 ml., add 0.04 gram of a mixture of equal parts of tannic and pyrogallic

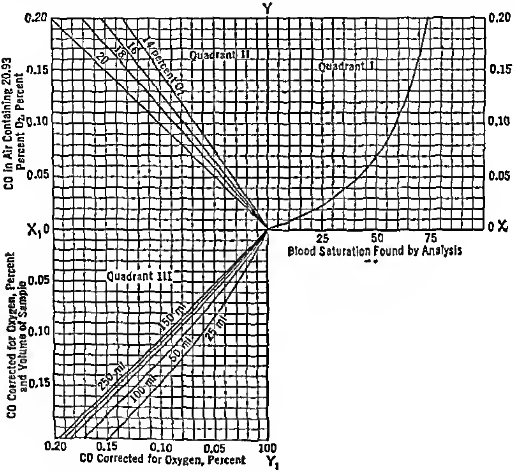

Fig. 40

Curves for Calculation of Percentages by Volume of Carbon Monoxide in Gas Samples From Percentage Saturation of Blood

acids. Gently invert several times to insure thorough mixing and compare with standards after 25-30 minutes. It is most convenient to read the final value from the graphs in Figure 40. If the oxygen percentage is 19.0-20.9 per cent, the volume of sample 250 ml. or more, and the analysis at 18-23°, read the carbon monoxide directly from quadrant I. Thus if the percentage saturation is 50, take this on the *OX* axis and follow the vertical line upward until it intercepts the curve, then right to the value. If the sample contains less than 19 per cent of oxygen, such

as 18 per cent, follow left from the value in quadrant I until it intercepts the line in quadrant II representing the oxygen percentage, then down to get the corrected percentage on the base line. The example then reads 0.07 per cent. If the sample is less than 250 ml., and previous instructions are followed into quadrant III to intercept the line in that quadrant representing the volume, follow left to the corrected carbon monoxide content. For a 50-ml. volume the preceding example becomes 0.077 per cent. Thus a correction may be applied for either oxygen content or volume of sample or both.

Standards. Draw 5 ml. of normal blood and add 0.025 gram of potassium citrate if the blood is to be used immediately, or 0.01 gram of sodium fluoride if the blood is to be kept 3 days or more. Divide into equal parts. Dilute a 2.5-ml. portion to 25 ml. with distilled water. Saturate the other portion with 3-5 per cent carbon monoxide gas and dilute to 25 ml. with water. From these prepare a series of mixtures containing 0, 10, 20, 30, etc., to 100 per cent carbon monoxide-hemoglobin. Put 1 ml. of each into narrow test tubes. To each tube add 1 ml. of a mixture of equal parts of freshly prepared 2 per cent pyrogallic acid solution and 2 per cent tannic acid solution. Mix and pour a little melted paraffin on the surface at once, keeping the tube in cold water. When the paraffin has solidified, place a disc of cardboard on the paraffin and fill with sealing wax. Avoid enclosing any air. The color develops in 25-30 minutes and lasts several weeks.

Carbon Monoxide by Ammoniacal Silver Nitrate Solution

A Pyrotannic Detector sold by the Mines Safety Appliance Co. of Pittsburgh is based on this reaction and is calibrated both in terms of air and ethylene as the basic gas. An accompanying scale reading is supplied.

Sample. *Air.* Aspirate the air to be examined through a soda-lime tube into a 250-ml. sample bottle.

Ethylene.[22] Concentrate the sample by distillation to bring it within the range of sensitivity of the method Take the sample of ethylene containing carbon monoxide in a small pressure cylinder. The fractionating apparatus as shown in Figure 41 is of glass with fused joints, with

[22] Harold S. Booth and Madeline B. Campbell, *Ind. Eng. Chem., Anal. Ed.* 4, 131-4 (1932).

the exception of pressure tubing connections T and C to the pressure cylinder and to the sample bottle.

Bulbs J and G are for condensation of the gas by liquid air in a Dewar flask. Mercury safety manometers K and H show the pressure in J and G. Sample bottle A has a capacity of 300 ml., of which the pressure is shown by the safety manometer E. An additional manometer L serves to test the efficiency of the rotary oil pump connected at P for evacuation of the system.

To operate, put the cylinder in place at T. Heat this with hot water to a temperature well above the critical temperature of ethylene. Close all stopcocks on the evacuated system to separate the fractionating bulbs and the sample bottle. Raise a Dewar bottle of liquid air around J and open stopcock S and valve V. The ethylene in the cylinder is all in gaseous form and is representative of the contents of the cylinder. When solid material equivalent to 25 ml. of liquid ethylene has condensed in J, close the valve and stopcock S. Allow the ethylene in J to liquefy. When the liquid begins to boil, open stopcocks N, D, and B to collect a sample of gas in the bottle A. When atmospheric pressure is reached, as shown by manometers K and E, close B and D and either immerse J in liquid air to prevent further rise in pressure or vent it to the air through O and P.

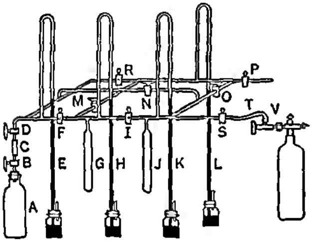

Fig. 41
Apparatus for Fractionation of Carbon Monoxide From Ethylene

All the carbon monoxide of importance in 25 ml. of a commercial grade of ethylene is contained in the first sample taken. If desired, a second similar bottle may be filled at A as a safety precaution. To complete, follow the directions which accompany the equipment.

Carbon Monoxide by the Molybdenum Blue Reaction

This reaction is one well known in the determination of phosphorus and several other materials. The fundamental reaction is discussed under phosphorus (page 660). As applied to carbon monoxide it is

believed that acid palladium chloride is reduced and in turn serves to reduce phosphomolybdic acid.[23] The function of the acetone is to accelerate the reduction of palladium chloride by carbon monoxide.[24]

Over the range of maximum sensitivity, up to 0.06 per cent, it is accurate to better than 10 per cent. As little as 100 ppm. or 0.001 per cent can be detected. The color is standardized by natural standards and may then be read photometrically in terms of transmittance. Since the method is purely empirical, its successful application depends on standardization of conditions. Satisfactory checks are given with other methods.

Other reducing gases will cause a corresponding result. The relative effects of several possible contaminants are ethylene 0.7, hydrogen sulfide 0.7, acetylene 0.5, hydrogen 0.03, gasoline 0.02-0.04, sulfur dioxide 0.0018, ammonia 0.0005, natural gas 0.00004, benzene 0.000025, ethanol, 0.000017, methanol 0.000017. All of these except hydrogen can be removed by suitable pretreatment. A yellow silica gel impregnated with a complex silico-molybdato catalyzed with palladium turns to green and bluish green in the presence of carbon monoxide.[25] As provided, the glass tube is about 125 mm. long. For use, break off the ends and aspirate the gas through it with a bulb and compare with a series of standards.

Reagents. Dissolve 0.5 gram of chemically pure palladous chloride, $PdCl_2$, in 2 ml. of concentrated hydrochloric acid and approximately 100 ml. of distilled water by heating in a covered beaker until solution is complete. Transfer to a 250-ml. volumetric flask with water, add 3 5 ml. of concentrated hydrochloric acid, and dilute to volume when cool. Dissolve 5 grams of chemically pure phosphomolybdic acid, 20 $MoO_3 \cdot 2H_3PO_4 \cdot 48\ H_2O$, in distilled water and dilute to 100 ml. Dilute concentrated sulfuric acid by adding it to 5 volumes of water.

Prepare the reagent by mixing equal volumes of the palladium chloride solution, phosphomolybdic acid solution, and 1:5 sulfuric acid. Let this stand at room temperature for 48 hours before use or heat at 60° for 4 hours. This reagent remains colorless in a refrigerator but darkens on long standing at room temperature. A blank to compensate for darkening is permissible.

The acetone to be used with this must be checked. Chemically pure acetone will usually be satisfactory without preliminary treatment. If

[23] R. D. Polis, L. B. Berger and H. H. Schrenk, *U. S. Bur. of Mines, Repts. Investigations* 3785, 13 pp. (1944).

[24] South Metropolitan Gas Co., *J. Soc. Chem. Ind.* 57, 79 82 (1938).

[25] *Technical News Bulletin of National Bureau of Standards*, No. 354, 73 (1946); Martin Shepherd, *Anal. Chem.* 19, 77 81 (1947).

the acetone causes a significant amount of color with an equal volume of the reagent, add a few drops of strong potassium permanganate solution and either reflux for an hour or let stand for 48 hours. In either case remove the moisture with anhydrous potassium carbonate and distill.

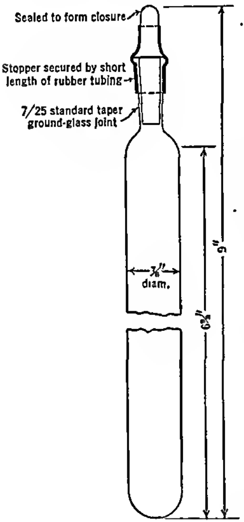

Fig. 42
Tube for Carbon Monoxide Determination

Sample. A 50-ml. glass-stoppered tube is required such as is shown in Figure 42. This is used for both development and reading of the color. The gas sample may have been taken by any conventional method. Transfer to the tube by water displacement, a convenient technic being indicated in Figure 43. If the carbon monoxide content is suspected of exceeding 600 ppm., which is 0.06 per cent, dilute the sample with carbon monoxide-free air by transfer of a limited, known volume to the tube before introduction of the sample. Drain excess water from the inverted tube after filling by momentarily removing the stopper and contacting the tube with a towel.

Procedure. Add 3 ml. of the mixed reagent to the sample tube and at the same time to a similar tube of carbon monoxide-free air. At once add 3 ml. of acetone to each. Carry these operations out rapidly to avoid loss of some of the sample. Fix the stopper in place as shown in Figure 42.

Place the tubes in a device designed for rotating them at 10 rpm. in a water bath maintained at $60° \pm 1°$. Rotate for exactly 60 minutes and remove. A color is developed which is a function of the time of rotation and temperature. Thus the color will continue to increase at a lesser rate on further rotation. If removed at the specified time and cooled promptly, the color is essentially constant. Clean the exterior of

each tube by immersing to the neck in chromic acid cleaning solution, wash, and dry before cooling.

Read the color of the sample and either compare with standards developed at the same time or with a transmittance curve. In the former case subtract the color value indicated by the blank; in the latter case correct for it directly by setting the instrument for 100 per cent transmittance with the blank in place. A suitable color filter is one covering the range 635-720 mμ. In that range the system follows Beer's law.

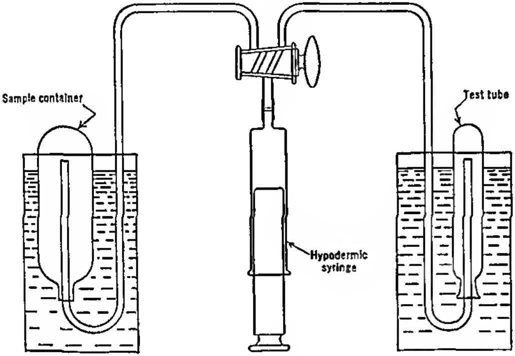

Fig. 43

Diagrammatic Sketch of Apparatus for Transferring Sample From Sample Container to Test Tube

Miscellaneous

A complex system for measurement of carbon monoxide in blood is keyed to the use of the step photometer.[26] A fresh 0.1 ml. sample diluted to 10 ml. with 0.1 per cent sodium hydroxide solution is divided into halves. One is reduced with sodium hyposulfite, $Na_2S_2O_4$, and the difference read with a mercury lamp and a 578-mμ filter. The other half is read at 546 mμ, interposing a gray glass. The ratio of the value at 578 mμ to that at 546 mμ is normally 0.41 but becomes zero with 100 per cent

[26] Ranke and Seydel, *Veröff Heeresssan. wes.* **108**, 200-10 (1939); *Z. Untersuch. Lebensm.* **81**, 66-7 (1941).

carbon monoxide-hemoglobin. The linearity of this is applied with an accuracy of about 3 per cent.

A method parallel to that with palladium chloride [27] uses ferric chloride which is reduced to the ferrous condition by the carbon monoxide in the presence of such catalysts as platinum sponge and silica gel. Reaction to only 50 per cent is common. The ferrous ion then by reaction with potassium ferricyanide gives a blue coloration which will detect 200 ppm.

[27] S. M. Chumanov and M. B. Akselrod, *J. Applied Chem.* (U.S.S.R.) 11, 720, 1236-7 (1938); *ibid.* 12, 1568-70 (1939); *Chimie et Industrie* 42, 471 (1939).

CHAPTER 64

CARBON DIOXIDE AND CARBONATES

Carbon dioxide, aside from the normal content in the air, is important in combustion gases. It is an impurity in any commercial cylinder gases. It is liberated from carbonates by acid and, if diluted in a known volume of air, may be estimated.

In general, passage of dilute carbon dioxide into a solution of sodium carbonate until equilibrium is reached will convert sufficient of the carbonate to bicarbonate so that the alteration in pH can be detected. Alternatives are turbidity as barium carbonate or by less direct means.

Sample

The sample for these methods will normally be a gas. The presence of any other gas altering the pH will interfere. The usual source of such interference is sulfur dioxide. Where such fumes or alkaline ones do interfere, methods of removal are given. Solid samples are used directly by some methods.

Standard

Outdoor air always contains approximately 0.031 per cent of carbon dioxide. Similarly the breath normally contains 4.38 per cent of carbon dioxide. For more concentrated standards make suitable mixtures of cylinder gas with air.

Carbon Dioxide by pH

When a gas containing carbon dioxide is brought into equilibrium with sodium bicarbonate solution, the pH level is a measure of the concentration of carbon dioxide in the gas.[1] The method has been well developed for natural and artificial color standards, reading of transmittance and for the glass electrode.

The amount of carbon dioxide dissolved depends on its partial pressure in the sample of air and is independent of the volume of air blown

[1] H. L. Higgins and W. M. Marriott, *J. Am. Chem. Soc.* 39, 68-71 (1917); P. W. Wilson, F. S. Orcutt and W. H. Peterson, *Ind. Eng. Chem., Anal. Ed.* 4, 357-61 (1932); P. W. Wilson, *Science* 78, 462 3 (1933).

through, after equilibrium is attained. Various indicators have been used and the conditions modified according to the expected concentration of carbon dioxide. Buffers as prepared for pH determination (Vol. 1, page 175) are suitable for preparation of standards. The method is inapplicable in the presence of fumes which would alter the pH, that is acid, ammonia, or amine fumes, unless the gas is scrubbed without absorption of carbon dioxide, as for example with 1 per cent sulfuric acid.

When applied by visual or photoelectric transmittance methods, it merely applies a newer technic of pH measurement. This has been successful visually, using filters of 580 mμ or 507-562 mμ.[2] A photoelectric colorimeter has been designed for use with methyl red for this purpose,[3] the sample cell being arranged for continuous flow of the test gas, except during momentary interruptions for reading. It was found desirable to use Corning No. 397 and Wrattan No. 74 filters. The carbon dioxide must never lower the pH below the pK value of the indicator, which for methyl red is 5.1.[4] Calculation indicates accuracy to be about 6 times as great with the photoelectric method as with the usual colorimetric method. There is no reason why any good photoelectric instrument applied to determination of pH should not be substituted and give the same accuracy. Another method[5] with the photoelectric colorimeter uses 0.0001 *M* sodium bicarbonate and one-fifth that amount of dibromothymolsulfonthalein. The pH is determined and applied without correction by use of the formula pH = 3.94 = 0.85 log P (18°) in which 3.94 is a constant found experimentally over the range 0.03-0.30 per cent carbon dioxide by volume. As a variation the solution not only contains 0.02 per cent of sodium hydroxide but also 1.75 per cent of sodium chloride.[6]

One special application is determination of organic vapors in air.[7] The vapors are combusted by passing over copper oxide, cooled, and the carbon dioxide equilibrated with sodium bicarbonate solution. In this case bromothymol blue was used. Another special case is bubbling of anesthetic gases through aqueous solutions of pH indicators to indicate the carbon dioxide content and therefore the necessity of renewal of soda

[2] Iwao Tisiro, *Mitt. med. Akad. Kioto* **26**, 163-72 (1939).

[3] Richard J. Winzler and J. Percy Baumberger, *Ind. Eng. Chem., Anal. Ed.* **11**, 371-5 (1939).

[4] For pK values of other indicators see Vol. 1, Page 183.

[5] Bycichin and Láska, *Chem. Listy* **29**, 201-2 (1935).

[6] Enzo Boeri, *Bull. soc. ital. biol. sper.* **18**, 284-5 (1943).

[7] Y. Kauko and T. Yli-Uotila, *Suomen Kemistilehti* **9B**, 3-4 (1936).

lime or other absorber.[8] When the level reaches 0.3 per cent it produces a yellow color with bromocresol purple. With methyl red the levels are 1 per cent, pale pink; 3 per cent, light red; and 5 per cent, deep red.

The methods are for amounts of 0.03-6 per cent and for amounts in the range 2-100 per cent.

Concentrations of 0.03-6 Per Cent. Prepare a 0.0667-molar monopotassium phosphate solution by dissolving 9.078 grams of pure recrystallized salt, KH_2PO_4, in distilled water. A solution of the salt should show no test for chloride or sulfate. The loss in weight at 20-30 mm. and 100° for 24 hours should be less than 0.1 per cent. On ignition the loss should be 13.23 ± 0.1 per cent. Add 200 ml. of 0.01 per cent phenolsulfonephthalein indicator solution and dilute to 1 liter. Standardized indicator containing sodium bicarbonate is not suitable.

Make a 0.0667-molar disodium phosphate solution by exposing to air, protected from dust, the pure recrystallized salt, $Na_2HPO_4 \cdot 12H_2O$, for 10 days or 2 weeks. The dihydrate, $Na_2HPO_4 \cdot 2H_2O$, is obtained. As a check on the purity of this, the solution should be clear and give no test for chloride or sulfate. The salt when dried at 20-30 mm. and 100° for 24 hours and carefully ignited should show a loss in weight of 25.28 ± 0.1 per cent. Dissolve 11.876 grams of this in water, add 200 ml. of 0.01 per cent phenolsulfonephthalein indicator solution, and dilute to 1 liter.

Mix the two standard phosphate solutions in the proportions indicated below:

Standard Value	*3*	*7*	*10*	*15*	*20*	*30*	*40*	*50*	*60*
KH_2PO_4 in ml. ...	5.4	10.5	15.0	20.0	29.0	33.0	40.5	46.5	52.0
$Na_2HPO_4 \cdot 2H_2O$ in ml.	94.6	89.5	85.0	80.0	71.0	67.0	59.5	53.5	48.0

Put these solutions into small test tubes, 10 by 75 mm., stopper, and seal.[9] Keep them in the dark when not in use. A small amount of thymol or toluene added to each solution prevents the growth of mold.

Prepare two standard solutions of sodium bicarbonate: 0.001 *N* and 0.0107 *N*. For the more dilute take 10 ml. of 0.1 *N* sodium carbonate solution. Add 200 ml. of indicator solution and dilute to 1 liter. For the more concentrated take 107 ml. of 0.1 *N* sodium carbonate solution. Add 200 ml. of indicator solution and dilute to 1 liter. Pass carbon

[8] Wm. B Draper and Bernard B. Langwell, *Colorado Med.* 32, 899-900 (1935).

[9] The standard solutions and apparatus as shown in Figure 44 may be obtained complete from Hynson, Westcott and Dunning, Baltimore, Md.

dioxide from a cylinder or from the lungs through these solutions in order to convert to sodium bicarbonate. This conversion need not be quantitative.

Put 5 ml. of the more dilute standard solution of sodium bicarbonate in a test tube provided with an inlet tube drawn to a fine capillary. Blow in the sample with an atomizer bulb or aspirate through the solution until no further color change occurs. This takes about one minute. Stopper the tube with a paraffined cork and compare the color imme-

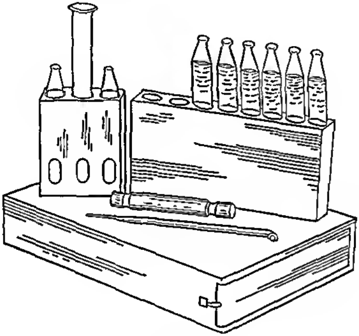

FIG. 44
Commercial Unit for Determination of Carbon Dioxide in Breath
(Hynson, Westcott & Dunning)

diately with the buffer tubes or with color discs which have been calibrated against natural standards. If off the scale, repeat with the more concentrated sodium bicarbonate solution.

By matching the sample against buffer solutions, one may read the standard value directly as parts of carbon dioxide per 10,000, or hundredths of a per cent, when the more dilute standard bicarbonate solution is used. When the more concentrated standard bicarbonate solution is used, the standard values represent parts per 1000 or tenths of a per cent. For the analysis of carbon dioxide higher than 6 per cent, an even more concentrated carbonate solution is required. The solutions are valid for temperatures of 20-25° and pressures of 730-800 mm. Adjust to within that range before reading. Properly conducted, an accuracy

to 5 per cent is obtainable, the errors being more in the collection of the sample than in the method of analysis. As outdoor air always contains approximately 0.031 per cent of carbon dioxide, the more dilute solution is easily checked using that as standard gas.

By Calculation. Another method gives the results by calculation from the pH of three solutions.[10] In the equation

$$\mathrm{pH} = -n[\log(Kk_{\mathrm{gas}}P) + c],$$

K is the primary ionization constant of carbonic acid, k_{gas} is the solubility factor of carbon dioxide, P is the carbon dioxide tension in terms of atmospheres, n is the rate of change of pH with change in $-\log\ (Kk_{\mathrm{gas}}P)$, and c is a factor dependent on the sample. The value of K is 3.50×10^{-7} at 25° and 3.12×10^{-7} at 18°.[11] The solubility of carbon dioxide is 1 volume at 1 atmosphere pressure within the accuracy of the method. However a simpler form of the equation permits elimination of some factors. In the derived form $\mathrm{pH} = -n(\log P + c)$, and since both n and c are unknowns two equations are necessary. These must be at two different carbon dioxide tensions with temperature constant.

Obtain the pH values[12] of the following solutions at the same temperature.

1. The original sample.
2. The original sample brought into equilibrium with the carbon dioxide of the atmosphere.
3. The original sample brought into equilibrium with the breath of the observer.

From 2 and 3 above calculate the values of n and c. Representative partial pressures of carbon dioxide in atmospheres are 0.00031 in atmospheric air and 0.0438 in air expired from the lungs. Substitute in the equation and solve for P, using the pH of 1 above. All values are absolute and the usual colorimetric pH standards may be used.

The principal errors are in reading the pH, variations in composition of atmospheric and alveolar air, and incomplete equilibria with air. The value in expired air is particularly subject to substantial variation with exercise, physical condition, and other factors. The accuracy can be

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[10] E. B. Powers and J. D. Bond, *Ecology* **9**, 364-6 (1928).

[11] Yrjö Kauko and Julius Carlberg, *Z. physik. Chem.* A173, 141-9 (1935); *Z. anal. Chem.* 102, 393 407 (1935).

[12] See Vol. 1, Chapter 21.

increased by using a known carbon dioxide mixture in place of the breath.

Concentrations of 2-100 Per Cent. Larger quantities of carbon dioxide are estimated by the effect on an equivalent mixture of phenolphthalein and sodium hydroxide.[13] An error of 0.1-0.2 mg. of carbon dioxide occurs from the air enclosed in the absorption flasks. This is not significant for amounts of carbon dioxide over 5 mg. Other possible acid vapors are absorbed by 1 per cent sulfuric acid. With the

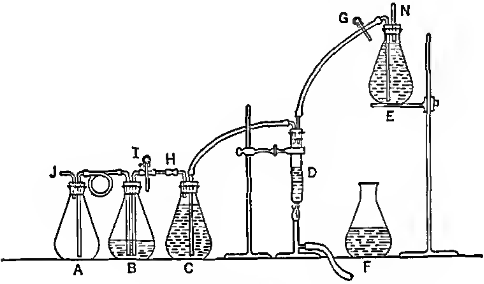


FIG. 45
Apparatus for Collecting Carbon Dioxide for Estimation With Alkaline Phenolphthalein

sample containing over 10 mg. of carbon dioxide the error is less than 3 per cent and the majority of estimations were within 1 per cent. The special apparatus used is shown in Figure 45.

Weigh or measure an amount to give between 10 and 100 mg. of carbon dioxide into tube D of the apparatus. In working with dilute gases pass a suitable volume of the gas through 30 per cent sodium hydroxide solution as an absorbent and use the solution so obtained or an aliquot as sample.

The absorbent must be selected according to the probable amount of carbon dioxide in the sample. The concentrations are given in

[13] E. M. Emmert, *J. Assoc. Official Agr. Chem.* **14**, 386-9 (1931); cf. Norman A. Spector and Barnett F. Dodge, *Anal. Chem.* **19**, 55-8 (1947).

Table 14. A normal solution of phenolphthalein contains 318 grams per liter.

TABLE 14. AMOUNTS OF SODIUM HYDROXIDE FOR USE WITH VARYING AMOUNTS OF CARBON DIOXIDE

Amount of Carbon Dioxide to Be Estimated (mg.)	*In 100 ml. Use the Following Weight of Phenolphthalein (g.)*	*In 100 ml. Use the Following Amount of Sodium Hydroxide* (g.)	*ml. N Solutions*
2-9	0.080	0.01	0.25
9-18	0.160	0.02	0.5
18-35	0.320	0.04	1.0
35-50	0.480	0.06	1.5
50-70	0.640	0.08	2.0
70-90	0.800	0.10	2.5
90-120	0.960	0.12	3.0

In preparation of the absorbing solution, dissolve more than the equivalent amount of phenolphthalein for 2 liters, in 1 liter of alcohol neutral to phenolphthalein. Dissolve the required amount of sodium hydroxide for 2 liters in distilled water or add the required amount of solution to water and dilute to 1 liter. Mix the two. For accurate work standardize by titration of a portion with standard acid.

Place the unknown sample in tube D, which may be varied in size according to the sample. Fill the tube to within 10-15 ml. of the top with water. Put 100 ml. of absorbent solution of the desired concentration in B. Fill C to within 10-20 ml. of the top with 1 per cent sulfuric acid. E and F contain 1:1 sulfuric acid.

At the start, stopcock I should be open and all connections airtight. Place the stopper of E in F. Blow in 10-15 ml. of sulfuric acid by opening G and at the same time blowing at N. Close G and replace the stopper and tubes in E without destroying the siphon. If there is a great deal of carbon dioxide in the sample, it may be necessary to introduce this acid slowly rather than all at once.

Heat D until the liquid has boiled for several seconds but has not boiled over into C. Withdraw the flame and immediately open the cock at G. This will sweep gases from D and C by 50 per cent sulfuric acid from E. When this reaches H, which is 100-125 mm. long to permit of ready observation, close I. Make sure that no acid goes through H to B. Disconnect B at H. Lower B below A and shake until B no longer changes color. If the solution becomes colorless or nearly so and there

is considerable solution in A, apply a suction at J. The vacuum produced when released will drive fresh solution back into B. If the amount of solution in B is small it may be decolorized several times. If both A and B become colorless, there is too much carbon dioxide for the amount of absorbing reagent used, or acid from C was carried over into B. To avoid the latter, the tubes at H and all connections must be free from acid before starting.

When no further color change occurs, remove the tubes from A and B, mix the solutions and compare with the color of a standard. Prepare this by similar treatment of a suitable volume of a solution containing 24.10 grams of sodium carbonate per liter of distilled water, equivalent to 10 mg. of carbon dioxide per ml.

Carbonate in Bicarbonate by pH

Except that the pH level of bicarbonate is raised rather than lowered by the impurity being measured, the method is similar to the estimation of carbon dioxide by passing it into sodium carbonate solution. Phenolphthalein is a suitable indicator.[14]

Procedure. Weigh 0.84 gram of sample and transfer at once to a 100-ml. volumetric flask. Add water to volume, stopper, and dissolve. As developed sample add 1 ml. of a 0.2 per cent phenolphthalein solution in 50 per cent ethanol to 25 ml. of the sample solution.

Prepare a preliminary standard by dissolving 0.53 gram of sodium carbonate in water and dilute to 100 ml. after adding 1 ml. of the indicator solution. As standard, dilute the required amount of this to 100 ml. with 0.53 per cent sodium carbonate solution and compare by balancing.

Carbon Dioxide Turbidimetrically as Barium Carbonate

Amounts of carbon dioxide up to 10 mg. are rapidly and conveniently estimated turbidimetrically by absorption in half-saturated barium hydroxide solutions.[15] Although designed for traces of carbonates in silicates, it is equally suitable for small samples of carbonates and adaptable to carbon by wet combustion of organic matter. Flushing out the system in advance with carbon dioxide-free air is not necessary if a blank is run.

[14] I. L. Degtyarev and M. I. Volynets, *Zavodskaya Lab.* 10, 582-6 (1941).

[15] Paul S. Roller and Guy Ervin, Jr., *Ind. Eng. Chem., Anal. Ed.* 11, 150-3 (1939).

The magnitude of the blank varies but little with concentration of the barium hydroxide solution.

The method was worked out for the Betz-Hellige turbidimeter (Vol. 1, page 120) with the milk glass in place when less an amount than 5 mg. of carbon dioxide was being determined, but removed when above that level. With a suitable blank obtained on the same day the calibration is a straight line and therefore is also satisfactory for measurement by transmittance. Because the blank also includes correction for the carbon dioxide in the air in the instrument, it would still have to be read and subtracted, setting the instrument at 100 with pure water.

The Hellige turbidimeter having a probable error of ± 2.0 per cent showed a probable error in the determination of ± 2.4 and ± 2.7 per cent with and without the filter. Therefore, the precision of the instrument is the controlling factor when the determination is properly manipulated.

The method is based on standardization of concentration of barium hydroxide, rate of air flow, time of boiling, and kind of acid used. None of these is critical. Moderate changes in concentration of the barium hydroxide are without effect. Rates of air flow up to 300 ml. per minute introduce no serious error; it should probably not fall much below 50 ml. per minute. The time of boiling will affect the opportunity for growth of the particles of precipitate. While hydrochloric acid is recommended, sulfuric acid can be used.

Apparatus. The required apparatus is illustrated in Figure 46. A is a wide-mouthed extraction flask of 150-ml. capacity which contains the sample. This flask is fitted with a three-holed stopper through which pass thermometer T, acid-dropping funnel F, and air inlet tube I. Air at about 5-cm. mercury pressure, entering through I, is freed of carbon dioxide by passing it successively through soda lime in tube L, 33 per cent potassium hydroxide solution in K, and saturated barium hydroxide solution in B. The rate of flow is regulated by stopcock S backed by an open or shut valve and is estimated by bubble counter U at the end of the train. The bubble counter also serves as a seal.

The inner tube of condenser C, which is fused to flask A, is 6 mm. in inside diameter and the jacket is 14 mm in inside diameter and 21 cm. long. Primary gas absorber E_1 is a 400-ml. wide-mouth Erlenmeyer flask into which gas-washing tube J_1 passes through a three-holed stopper. A second similar absorber may be placed in series with E_1 as a check on the completeness of the absorption but in operation proves unnecessary

Procedure. Transfer the weighted or measured sample of solid or liquid to flask A. Insert the rubber stopper and add boiled and cooled water until the flask is about two-thirds full. Transfer approximately 300 ml. of filtered, half-saturated barium hydroxide solution into the absorber E_1 by connection G_1. When this has been completed, close stopcock S_1 and the upper screw clamp on G_1.

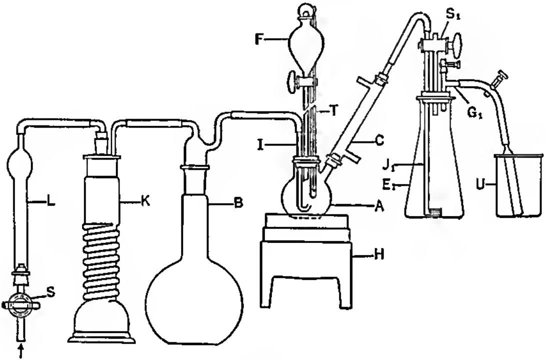


FIG. 46
Apparatus for Carbon Dioxide Absorption

Add 10 ml. of concentrated hydrochloric acid to the sample through F. Heater H should have been previously brought to temperature out of contact with A. Put it in place to heat A as promptly as possible. Start the flow of air through the system L, K, B, A, C, E_1, U at about 120 ml. per minute. As soon as the solution in flask A boils, reduce the heat so as to maintain gentle boiling.

After the solution has boiled for 6 minutes, remove heater H and stop the air flow. Transfer part of the suspension from E_1 to an instrument for reading. After 2-3 minutes in the instrument, the reading decreases but can be restored by shaking the suspension to break up flocculates. In 15 minutes the growth of particles has proceeded to such an extent that readings are unreliable. After use, rinse the apparatus, including

the turbidimeter cups, with dilute hydrochloric acid and then with water.

Carbonates by p-Nitrosothymol

The presence of carbonates in bicarbonates and of bicarbonates in carbonates can be estimated by their color reaction, in reality a pH function, with *p*-nitrosothymol.[16] The alkalinity of the carbonate produces a yellow color if only a trace is present and a red color of thymoquinone monoxime when larger amounts are present.

Excess of the reagent is added and that excess filtered off. The amount of excess is not significant. The method is applicable to potassium and lithium carbonates, although described for sodium carbonate. The presence of 0.001 per cent of sodium carbonate can be detected and the method is applicable up to 1 per cent. Intervals which can be distinguished range from 10 to 50 per cent.

Standards. Prepare a solution of *p*-nitrosothymol containing 6.3 grams per 100 ml. in neutral acetone. To Nessler tubes add volumes of 10.6 per cent sodium carbonate solution in graded amounts. A suitable preliminary series is 0.1, 0.3, 0.5, 1, 3.5, 10, 15 ml. If the range in which the carbonate will fall is known, a series at closer intervals is preferable. Dilute each tube to 15 ml. with water. To each tube add an amount of reagent solution equal to half the volume of sodium carbonate solution. Compensate in the series for the difference in volume of reagent added by adding acetone to the lesser ones to equal the maximum volume added. Dilute each tube to 25 ml. with water.

Shake the tubes for 10-15 minutes and filter out the excess reagent. The standards deteriorate but little in a few days but should be fresh for the highest accuracy. Standards should be protected from light.

Procedure. Place suitable volumes of samples in Nessler tubes, the volumes being less the greater the carbonate content. Dilute with water to 15 ml. Add an excess of the reagent as prepared for the standards. A large excess does no harm and will be filtered off later. No greater volume of reagent should be used than the maximum in the standards. Add acetone to equal the total volume in the standards. Shake for 10-15

[16] W. Taylor Sumerford, David Dalton and Robert Jordan, *Ind. Eng. Chem., Anal. Ed.* 15, 38-9 (1943).

minutes, filter, and compare. Usually after getting an approximate value it will be necessary to prepare a more closely spaced series.

CARBON DIOXIDE: CAPACITY OF PLASMA BY DIPHENYL SEMICARBAZIDE

Serum which has not been exposed to air may be saturated with carbon dioxide to determine its capacity. The series of reactions are then precipitation as barium carbonate, solution in acid and precipitation of the barium as chromate, and finally the familiar colorimetric determination of chromate with diphenylcarbazide.[17]

Sample. *Blood.* To avoid lactic acid in the blood the patient should be well rested before the sample is taken. Transfer 0.2 ml. of a solution containing 0.6 per cent of ammonium oxalate and 0.4 per cent of potassium oxalate to a centrifuge tube. Evaporate to dryness as the anticoagulant. Connect the tube with a centrifuge cup and fill both with low-viscosity mineral oil. Puncture the finger of the patient, submerge the finger in the oil, and collect 0.5 ml. of blood without contact with the air. Mix this blood sample with the anticoagulant using a fine glass rod. Pour off excess oil so that the tube is about one-third full and centrifuge. Transfer the plasma to another centrifuge tube and blow carbon dioxide through it until saturated. As an alternative, plasma from a larger sample which has been properly protected can be used.

Prepare a barium reagent containing 2.5 grams of barium hydroxide octohydrate and 50 ml. of concentrated ammonium hydroxide per 100 ml. To 1 ml. of water, which has been added boiling, and cooled in the stoppered tube, add 0.1 ml. of the plasma saturated with carbon dioxide and 0.2 ml. of the barium reagent. Mix, let stand for 5 minutes, and add 10 ml. of boiled and cooled water. Add about 0.5 mg. of diatomaceous earth, mix well, and centrifuge. Decant thoroughly and add 0.5 ml. of 1:2 hydrochloric acid to the precipitate. Heat in boiling water for 0.5 minute and cool quickly.

Prepare a chromate reagent containing 0.25 gram of ammonium chromate and 50 ml. of concentrated ammonium hydroxide per 100 ml. Add 1 ml. of this, mix, and add 8 ml. of water. Mix and centrifuge. Drain thoroughly and add 8 ml. of water and 1 ml. of concentrated hydrochloric acid. Mix well with a rod and rinse the tube and rod into a 100-ml. cylinder. Dilute to about 80 ml. and add 0.5 ml. of a 1 per

[17] William G. Exton, F. Schattner and A. R. Rose, *Am. J. Clin. Path.* 11, 632-42 (1941).

cent solution of diphenyl carbazide in 95 per cent ethanol. Dilute to 100 ml. and read against a standard equivalent to 100 volume per cent of carbon dioxide.

Standard. Dissolve 0.0882 gram of potassium chromate in water and dilute to 1 liter. To 10 ml. of this add 80 ml. of water, 1.0 ml. of concentrated hydrochloric acid, 0.5 ml. of the carbazide reagent, and dilute to 100 ml. This is the standard equivalent to 100 volume-per cent of carbon dioxide. Prepare lower standards by dilution of lesser amounts of the stock solution.

CHAPTER 65

CYANIDE

ALTHOUGH not nearly as poisonous as popularly supposed, cyanides much above 100 ppm. are dangerous in gases being respired, and their presence in solid foods must be controlled. The wide use in fumigation of foodstuffs leads to a need for sensitive methods. Forensic chemistry necessarily includes such methods. The principal methods are by oxidation of the colorless form of a phthalein indicator, by conversion to a thiocyanate which is then estimated as the ferric complex, as Prussian blue, and by the reaction of cyanogen bromide with pyridine.

SAMPLES

Gas.[1] By absorbing the cyanide in alkaline solution as described for vaporization of cyanides from organic samples, satisfactory results are obtainable with as little as 0.001 mg. per liter of air.

Inorganic. If the sample is an inorganic solution render slightly alkaline with sodium hydroxide solution and dilute to a suitable volume according to the cyanide content. In dissolving a salt to be analyzed for cyanides, the solution must be kept alkaline to prevent loss. This inorganic solution is then used directly. In either case filter if not already clear. If interfering substances are present or if the solution is very dilute, concentrate by volatilization as described for organic samples with low cyanide content.

Water.[2] Transfer 500 ml. of filtered sample to a flask. If sulfides are present, add an excess of a lead salt. Add sufficient tartaric acid to neutralize and 0.5 gram in addition. Distill, collecting 25 ml. if less than 0.1 ppm. of cyanide is present, otherwise 50 ml.

Organic. *Cyanide High.* Macerate 50 grams of finely ground material with 100 ml. of water. Wash into a distilling flask with another

[1] Julius Meyer and Hsue-Wen Fan, *Gasmaske* 11, 17-18 (1939).
[2] A. E. Childs and W. C. Ball, *Analyst* 60, 294-9 (1935).

100 ml. of water. Connect with a condenser dipping into 50 ml. of 4 per cent potassium hydroxide solution. Fit the distilling flask with a separatory funnel containing 50 ml. of concentrated sulfuric acid. When the apparatus is set up and all connections are tight, add the acid to the sample and distill over about 150 ml. Dilute the distillate to a convenient volume, such as 250 ml., and take an aliquot.

Cyanide Low.[3] Isolate and concentrate the cyanide by aeration. The apparatus is shown in Figure 47. The aeration flask is a 300-ml. conical

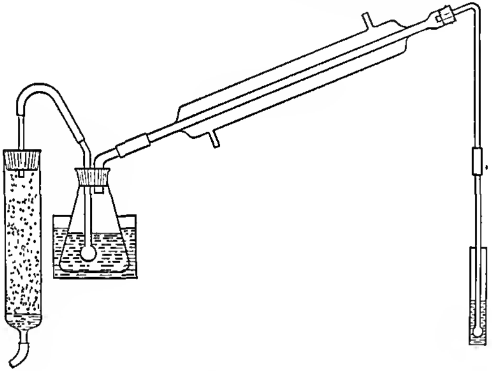
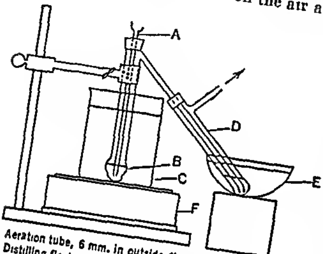


FIG. 47
Apparatus for Concentrating Hydrocyanic Acid by Aeration

one, fitted with a 2-holed rubber stopper. Through one hole insert a tube extending nearly to the bottom of the flask and having a bulb with several small holes at its lower extremity. Through the other hole insert a glass exit tube connected with a condenser as shown. The inlet tube is connected through a soda-lime tower with a source of compressed air. From the upper end of the condenser a glass tube extends downward to another bulbed tube like that used in the aeration flask This is immersed in a glass tube as shown, such as a 6-inch test tube or flat-bottom glass tube.

[3] W. O. Winkler, *J. Assoc. Official Agr. Chem.* **22**, 349 55 (1939).

Transfer 11 ml. of water and 2 ml. of 10 per cent sodium hydroxide solution to the absorber, or for very small amounts of cyanide use only 1 ml. of the alkali solution. Adjust the flow of compressed air to deliver 800 ml. to 1 liter per minute. Grind the sample and weigh 25 grams into the flask. Add 180 ml. of 1:50 sulfuric acid and close with a solid rubber stopper. Shake for a few minutes to disintegrate the sample further. Remove the stopper, rinse it into the flask, and put in place in the apparatus. Immerse the flask in a 35 per cent glycerol bath to a depth of 1-1.5 inches. Turn on the air and heat the bath rapidly to 104°. Thereafter maintain the bath at 105-110°. Continue the air flow for 15 minutes after the bath reaches 100°. Remove the flame and turn off the air. Dilute the contents of the absorber to a known volume and take a suitable aliquot as sample. The method will recover 0.5-16 mg. of hydrocyanic acid with an accuracy within 2 per cent.



A. Aeration tube, 6 mm. in outside diameter
B. Distilling flask, 12 mm. in outside diameter by 120 mm.
C. Water bath
D. Receiving tube 12 mm. in outside diameter by 120 mm.
E. Ice bath
F. Hot plate

Fig. 48
Diagram of Microstill

Insects.[4] The method is designed for analysis of insects from fumigation. Set up a microstill such as is shown in Figure 48. Add the weighed charge of insects and 1 ml. of water. Put 1 ml. of 5 per cent sodium carbonate solution in the receiver. Add 5 drops of saturated tartaric acid to the still and connect to the receiver. Have the tip of the still just dip below the surface of the solution in the receiver. Immerse the receiver in ice water and the still in boiling water. Draw a vacuum and adjust the flow of air to 1 bubble per second. Distill for 20 minutes and remove the receiver. The contents are a sample for microdetermination by the Prussian blue method.

Materials Treated with Insecticides.[5] Wash the sample thoroughly with water. Add 4 per cent sodium hydroxide solution to strong alka-

[4] Robert A. Fulton and Mildred J. Van Dyke, *Anal. Chem.* 19, 922-3 (1947).
[5] M. M. Raines and A. I. Krupkin, *J. Applied Chem.* (U.S.S.R.) 10, 960-2 (1937).

linity and evaporate to 1-2 ml. Use this as sample for development of color as ferrocyanide. If necessary because of impurities, separate the cyanide by aeration.

Flaxseed.[6] Grind 25 grams of seed in a steel mortar and macerate for 45 minutes at 45° with 500 ml. of water. Follow the method for organic samples high in cyanides but add 5 ml. of concentrated hydrochloric acid and distill. Then add 50 ml. of concentrated hydrochloric acid and distill again. Combine these distillates for analysis of an aliquot.

Standard

Dissolve 0.2408 gram of potassium cyanide in water and dilute to 1 liter. This contains 0.1 mg. of hydrogen cyanide per ml. For the more important methods special standards are given after the procedure, the use of which simplifies the determination.

Cyanides by Phenolphthalin

This color development depends on a rather complex series of reactions.[7] The cyanide in the presence of an excess of a cupric salt forms insoluble cuprous cyanide quantitatively, thus reducing the copper. Simultaneously phenolphthalin, the reduced form of phenolphthalein, is oxidized to the indicator form which will therefore give the usual color in alkaline solution.[8]

The ferricyanides and free halogens give the same reaction; hence it is usually applicable only to cyanides which have been concentrated by volatilization. Sulfides interfere but can be fixed by addition of a lead salt. Ferrocyanides, chromates, nitric acid, ferric chloride, and salts of the halogens do not interfere. The color fades quickly in aqueous solution. The color in 30 per cent ethanol is almost as intense as in water and remains unchanged for at least an hour. The system deviates somewhat from Beer's law. The technic can be refined to determine 0.0002 mg. of hydrogen cyanide in 10 liters of air, or 0.02 ppm.

[6] Juan F. Saredo, *Ph* 8, No. 2, 16-42 (1936); *Anales asoc. quím. farm Uruguay* 42, 29-35 (1939).

[7] Wilbur A. Robbie and P. J. Leinfelder, *J. Ind. Hyg. Toxicol.* 27, 136-9 (1945).

[8] F. Weehuizen, *Pharm. Weekblad* 42, 271 (1905); A. E. Childs and W. C. Ball, *Analyst* 60, 294-9 (1935); W. O. Moffitt and E. H. Williams, *ibid.* 62, 101-7 (1937); W. O. Winkler, *J. Assoc. Official Agr. Chem.* 22, 349-55 (1939); *ibid.* 24, 380-3 (1941); Wilbur A. Robbie, *Arch. Biochem.* 5, 49-58 (1944).

ides, phosphates, arsenates, iodates, oxalates, tartrates, and citrates interfere slightly. Reasonable amounts of chlorides, bromides, and iodides are without effect. The concentrations of ferric ion and chloride in sample and standard must be closely controlled.[13] The color is read satisfactorily by photoelectric measurement of transmittance and conforms to Beer's law.[14] The blank is rather high. Accuracy to 0.4-4.0 per cent is obtained.

Procedure. Transfer the sample solution to a 250-ml. beaker and add 3 ml. of a 15 per cent solution of sodium sulfide. Evaporate to dryness on a steam bath and heat for about 10 minutes after it is dry. Wash down the sides of the beaker with a few ml. of water and repeat the evaporation and baking.

Add 8 ml. of distilled water and dissolve the residue. Add 1:3 acetic acid until acid to litmus and about 0.2 ml. in excess. Again evaporate to dryness on the steam bath. Add 5 ml. of water, mix well with a policeman to work over the residue, and filter through a heavy asbestos mat on a Gooch crucible, with a 50-ml. volumetric flask also marked at 48 ml. as receiver. In this it is convenient to use a filtering bell jar. Wash the beaker and mat with two 5-ml. portions of water. Finally wash the funnel with 2 ml. of water. The filtrate must be no more than faintly opalescent. When difficulties are encountered in filtration,[15] extract the evaporated residue with 10 ml. of absolute acetone before extraction with water.

Add 3 ml. of 1:1 nitric acid, then dilute with ethylene glycol monomethyl ether—methyl Cellosolve—to 48 ml. Add 1 ml. of a 10 per cent solution of ferric ammonium sulfate, $FeNH_4(SO_4)_2 \cdot 12H_2O$, dilute to volume with the organic solvent, and mix. Let stand for 20 minutes and compare with standards or read the transmittance in a 2-inch cell using a filter having its maximum transmission around 490 mμ. The color fades rather quickly. This is probably due to reduction by isodithiocyanic acid, hydrocyanic acid, and hydrogen sulfide. Sample and standards should therefore be developed at the same time and compared promptly.

As standards, add 1-10 ml. of the standard thiocyanate solution at 1-ml. intervals to 50-ml. volumetric flasks. To these add water, the approximate volume of 1:3 acetic acid required by the sample less 0.2 ml., and sufficient sodium or potassium hydroxide to neutralize to litmus.

[13] Juan F. Saredo, *Ph* 8, No. 2, 16-42 (1936); *Anales acos. quím. farm.* (Uruguay) 42, 29-35 (1939).

[14] W. O. Winkler, *J. Assoc. Official Agr. Chem.* 24, 380-3 (1941).

[15] Maxwell O. Johnson, *J. Am. Chem. Soc.* 38, 1230-5 (1916).

Dilute to 17 ml. with water and complete as in the case of the sample, starting at "Add 3 ml. of 1:1 nitric acid . . ." Run a blank containing all the reagents, including any present from preparation of the sample. The value can be expected to be of the order of 0.05-0.015 mg. of hydrocyanic acid. In methods other than transmittance, determine this by dilution or balancing from the lowest of the standards.

Standard. Prepare a standard solution by dissolving 15 grams of potassium thiocyanate in water and diluting to 1 liter. Standardize gravimetrically with silver until 1 ml. contains 14.92 mg. of potassium thiocyanate. This is equivalent to 10 mg. of potassium cyanide per ml. Dilute 10 ml. of this to 1 liter before using with the ferric chloride reagent, thus making each ml. of the final color equivalent to 0.1 mg. of potassium cyanide.

Cyanide as Cyanogen Bromide

Traces of cyanide may be converted in neutral or acid solution into cyanogen bromide by reaction with bromine-water. After removal of excess bromine with sodium arsenite, this is estimated in dilute pyridine solution by its reaction with an amine such as benzidine.[16] The same reaction can be obtained with chlorine to form cyanogen chloride but its boiling point of 13° renders the losses much greater than with cyanogen bromide boiling at 61° C. The iodide is unsuitable because it reacts only slowly with the reagent. The sensitivity of color development increases with decrease in pyridine in the aqueous reagent to 10 per cent but at 25 per cent is on a flat portion of the curve. The benzidine hydrochloride must be added separately as it darkens rapidly when standing in pyridine solution.

Excess bromine must be completely removed; it reacts with the reagent to give a blue color changing to brown. Excess of sodium arsenite removes this without otherwise affecting the color. The reaction with bromine is almost instantaneous. The color is intensely red and conforms to Beer's law. This color changes during the period from 6 to 24 minutes after mixing, but the fraction transmitted by a blue filter does not. Ferricyanides and cyanates have no effect. Oxidizing and reducing agents are removed by the treatment.

The minimum detectable is 0.00035 mg. and up to 0.003 mg. can be determined. Although thiocyanates give the same reaction, they are

[16] W. Konig, *J. prakt. Chem.* 69, 105 (1904); *Z. angew. Chem.* 115, 1905 (1904); W. N. Aldridge, *Analyst* 69, 262-5 (1944); *ibid.* 70, 474-5 (1945).

nonvolatile, permitting a difference method. The technic is most satisfactory by transmittance.

Procedure. *Thiocyanate Absent.* Dilute the sample so as to contain not over 0.001 mg. per ml. Acidify the neutral or alkaline solution with acetic acid or trichloroacetic acid. To a 2-ml. sample, add 0.2 ml. of saturated bromine-water. Shake well and then add 0.2 ml. of 2 per cent sodium arsenite solution. At this stage it is permissible to stopper the solution and let it stand for 2 hours. Mix well and blow out any bromine vapor in the tube.

Redistill pyridine with water, collecting the constant-boiling mixture around 93°. Add 3 ml. of this reagent and 0.6 ml. of a 5 per cent solution of benzidine in 1:50 hydrochloric acid. On mixing, an orange color develops at once and changes to red within 10 minutes. Compare after 15-20 minutes with a standard developed at the same time or read the transmittance around 604 mμ.

Thiocyanate Present. Make one determination as though thiocyanate were absent. Take another sample, acidify as usual, and pass air saturated with water vapor through it for 15 minutes. This volatilizes all the cyanide. Now develop the color as usual and determine. The difference between the first and second results is cyanide.

Cyanides by Picric Acid

Cyanides give the dark red color of the alkali salt of isopurpuric acid with alkaline picrate solution. In the absence of substances such as aldehydes, acetone, and hydrogen sulfide, giving a similar reaction, it is quantitatively applicable.[17] Beer's law is followed in the range 1.6-4.4 mg. per 100 ml. of developed solution. By proper adjustment it can be applied to determination of 0.08 mg. in 25 ml. with an accuracy of 2 per cent. Although the color develops slowly at room temperature, the maximum intensity is developed in less than 10 minutes at 100°. The color is unchanged after 2 months. A modification [18] of the method uses sodium picrate paper for absorption and subsequently extracts the color.

Procedure. *Direct.* Transfer 10 ml. of 1 per cent aqueous solution of picric acid and 1 ml. of 5 per cent sodium carbonate solution to a

[17] Knud O. Möller and Kristrim Stefansson, *Biochem. Z.* 290, 44-57 (1937).

[18] F. S. Nowosand and R. M. MacVicar, *Sci. Agr.* 20, 566-9 (1940); P. G. Hogg and H. L. Ahlgren, *J. Am. Soc. Agron.* 34, 199-200 (1942).

25-ml. volumetric flask. Allow for any sodium carbonate in the sample by reduction of this amount of sodium carbonate solution. Add 5 ml. of the sample cyanide solution, which must not be acid. Previously dilute the sample if necessary to fall in the range of 0.01-0.02 mg. per ml. In similar flasks with the same reagents add 0.25, 0.80, 1.20, and 1.60 ml. of standard hydrocyanic acid solution containing 0.1 mg. per ml. Prepare a blank with 1 ml. of water added in place of the sample. Heat the sample, standards, and blank for 12 minutes in a boiling water bath, then cool and dilute to volume. Compare sample and standard, deducting the effect of the picric acid in the blank. For determination of transmittance, use 530 mμ, which transmits the developed color but masks that of picric acid.

Extraction. This method is usually applied to plant materials, such as 0.15 gram of macerated sample in water. Place a neutral 10-ml. sample and corresponding standards in test tubes. Soak strips of filter paper, $10 \times 12 \times 0.5$ cm., in a solution of 2.5 per cent sodium carbonate and 0.5 per cent picric acid, and suspend from tight stoppers at room temperature for 24 hours. Approximations are obtainable from the colors of the strips so prepared. For comparison transfer the strips to test tubes containing 10 ml. of water. Let stand until the color is extracted and compare the colors directly or by balancing.

Cyanide as Potassium Ferrocyanide

Potassium ferrocyanide reacts with ferric salts to give ferric ferrocyanide, Prussian blue. Although this is insoluble it may be obtained so finely suspended as to appear to give a true solution and is then suitable for colorimetric comparison.[19] Alkaline salts of the halogens other than potassium fluoride are without effect. The latter is helpful. Reducing agents may interfere. To obtain increased sensitivity the Prussian blue may be developed on a filter paper disc by absorption and compared with standards. Thus applied it will detect 0.0001 mg. of hydrogen cyanide. The standards may be preserved under glass indefinitely. The special absorber used is shown in Figure 49. The size of orifice shown there may be altered for different sizes of samples. Thus for 0.0002-0.001 mg. of cyanide a 4-mm. orifice is suitable; for 0.001-0.005 mg. the 10 mm.

[19] E. Berl and M. Delpy, *Ber.* **43**, 1430-1 (1910); M. M. Raines and A. I. Krupkin, *J. Applied Chem.* (U.S.S.R.) **10**, 960-2 (1937); A. O. Gettler and L. Goldbaum, *Anal. Chem.* **19**, 270-1 (1947); Robert A. Fulton and Mildred J. Van Dyke, *Anal. Chem.* **19**, 922-3 (1947).

indicated in the figure is appropriate; whereas for 0.005-0.02 mg. of cyanide 15 mm. is preferable.

Procedure. *Development as a Dispersion.* To the alkaline sample containing not less than 0.2 mg. of cyanide in 2 ml. of solution, and to a suitable volume of standard cyanide solution, add 0.5 ml. of 0.5 per cent ferrous sulfate solution. For 1 mol of hydrocyanic acid at least 2 mols of ferrous sulfate are needed. After standing for 10 minutes with frequent shaking, heat to 60-80° for 2-3 minutes. Cool to room temperature and let stand for 5 minutes Add 3 drops of 0.1 per cent ferric chloride solution and 1:4 hydrochloric acid until acid, plus 0.25 ml. in excess. Heat until colorless and set aside until a blue color develops. Dilute to volume and compare, or read at 520 mμ and apply to a calibration curve.

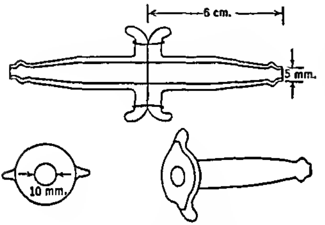


FIG. 49
Apparatus for Absorption of Cyanide as Prussian Blue

Development on Paper. To prepare the paper, soak filter paper in 10 per cent ferrous sulfate solution and suspend in the air to dry. Then dip in 20 per cent sodium hydroxide solution until thoroughly wet, and dry again. Cut to size for use with the units shown in Figure 49.

Place the sample containing 0.001-0.005 mg. of cyanide, diluted or concentrated to 5 ml., in an aeration tube connected with the absorption unit by rubber tubing. Place the aeration tube in a beaker of water at 90°, making sure that the heating water is not above the level of the liquid in the aeration tube. Apply suction for 5 minutes, during which all of the hydrogen cyanide is volatilized, absorbed by the test paper, and converted to Prussian blue. Remove the test paper and soak in 1:4 hydrochloric acid to dissolve the iron hydroxides which partially mask the blue stain. Wash the paper with distilled water and dry. Compare the stain with standards similarly prepared

CYANIDE AS A DYE FORMED WITH PYRAZOLONES

An extremely sensitive test for cyanides consists in converting the cyanide to cyanogen chloride with chloramine T and then reacting with

pyridine containing 1-phenyl-3-methyl-5-pyrazolone and bispyrazolone to form a blue dye.[20] The dye is stable at pH 7-9. Below 7 it changes progressively to a red, above 9 it slowly fades to orange. Excess chloramine T will bleach it. To have the dye stable the bispyrazolone is necessary. Over the range up to 0.0012 mg. of cyanide per ml., the system follows Beer's law.

Reagent. To prepare bis-(1-phenyl-3-methyl-5-pyrazolone) dissolve 17.4 grams of recrystallized 1-phenyl-3-methyl-5-pyrazolone in 100 ml. of 95 per cent ethanol and add 25 grams of freshly distilled phenylhydrazine. The insoluble product will have separated on refluxing for 4 hours. Filter and wash the residue with 95 per cent ethanol. For use dissolve 0.1 gram of the bispyrazolone in 100 ml. of pyridine and mix with 500 ml. of a saturated aqueous solution of 1-phenyl-3-methyl-5-pyrazolone. The pink color which develops on standing does not affect the final result, but the reagent should not be more than 3 days old.

Procedure. Dilute or concentrate a sample containing 0.0002-0.0012 mg. of cyanide to 1 ml. Add 0.2 ml. of 1 per cent chloramine T solution, stopper, and shake. After 1 minute, add 6 ml. of the prepared reagent solution and mix. After 20 minutes, read the transmittance at 630 mμ and compare with a calibration curve. The color is stable for at least 30 minutes after maximum development. The zero point should be set with a reagent blank.

Miscellaneous

Either acid or alkaline hydrolysis of hydrocyanic acid gives an ammonium salt. This must be carried out in a closed system to avoid loss. The ammonia can be estimated by Nessler's reagent and calculated to hydrocyanic acid.[21] Acid hydrolysis is preferable because alkali attacks the glass. The most convenient method of hydrolysis is with special glass autoclaves designed for work with urea[22] Satisfactory results can be obtained by sealing the samples in medicinal ampoules. The technique is described for that method. The sample nesslerized should not contain more than 0.5 mg. of hydrocyanic acid. If necessary an aliquot can be taken.

Transfer the alkaline aliquot to be used to a 35-ml glass ampoule. Cool in an ice bath. Add 5 ml of concentrated hydrochloric acid and

[20] Joseph Epstein, *Anal. Chem.* **19**, 273 4 (1947).
[21] Nathan Gale and Andrew J. Pensa, *Ind. Eng. Chem., Anal. Ed.* **5**, 80-1 (1933).
[22] S. L. Leiboff and B. S. Kahn, *J. Biol. Chem.* **83**, 347-52 (1929).

similar types of gases. Volatile amines, ammonia, hydrogen sulfide, hydrogen cyanide, and some unsaturated organic compounds will reduce the ferric iron if present when the solution is acidified, leading to low results. Preliminary scrubbing with acid and alkali is provided to remove all but unsaturates. They are avoided by evacuation of the flask before acidifying. The scrubbing may not eliminate peroxides. The size of the blank becomes serious when as little as 0.001 per cent of oxygen is pres-

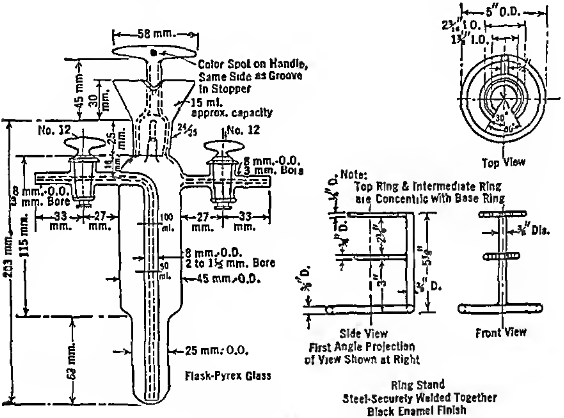


FIG. 50

Shaw Absorption Flask

ent. If the concentration exceeds 1 per cent and there is difficulty in dissolving ferric oxide, use more acid in known amounts, correspondingly increase the dilution, and adjust the acid addition to the solution on dilution.

Sample. The sample is assumed to be available on a sampling line by any well-known procedure. Charge a scruhber—a test tuhe will do—with 25 ml. of 1:4 sulfuric acid and connect to the sample line. Next charge another such scruhber with 30 ml. of 30 per cent potassium hydroxide solution. This amount of alkali is adequate for scruhbing 3 per cent of carbon dioxide from 140 liters, which is 5 cubic feet, of gas.

Purge the scrubbers with sufficient amount of gas sample at 28-56 liters per hour until at least 6 liters have been passed.

Prepare an absorption flask, the volume of which is accurately known, as by water displacement. Charge this with 15 ml. of a solution containing 15 grams of ferrous ammonium sulfate and 8.7 ml. of concentrated hydrochloric acid per liter. This solution should be less than 48 hours old or should have been stored in an inert atmosphere. Purge this flask with at least 14 liters of the gas sample. The scrubbers and absorption flask may be purged at the same time but the volumes of gas to be passed are additive. Remove the absorption flask from the sample line after closing the inlet and outlet. Relieve any pressure in the flask by opening the outlet valve momentarily. Take temperature and barometer readings at this time. Place 25 drops of 50 per cent sodium hydroxide solution in the flask without admission of air. If the Shaw flask is used, put this in the funnel neck, cool the enclosed gas with water, allow the sodium hydroxide solution to flow in slowly through the split part in the stopper, and wash the residual caustic solution in with two portions of 1-2 ml. of water.

Place the flask in a mechanical shaker and agitate vigorously for 1 hour; one-half hour has been found inadequate. A suitable agitator is a rod, carrying the flask, operated at a 45° angle by an eccentric having a 10-cm. stroke at 120 rpm. At the end of 1 hour evacuate thoroughly if unsaturated hydrocarbons are present. Any air leakage will still be important and in use of the Shaw flask it is desirable to have a few ml. of water in the funnel top to detect leakage. Otherwise it is only necessary to chill the flask with cold water. Add exactly 10 ml. of concentrated hydrochloric acid by the same means used for adding sodium hydroxide solution. Wash this in with two 1-2 ml. portions of water and shake sufficiently to obtain thorough mixing. Minor air leakage is now unimportant. If the ferric hydroxide does not dissolve readily, place the flask in hot water, venting the flask to the air by a suitable means if it has not been evacuated.

When all the iron is in solution, cool the contents of the flask and wash into a volumetric flask of suitable size. If this flask is 100 ml., add 0.7 ml. of concentrated hydrochloric acid; if more than that, add 8.7 ml. for each additional 100 ml. of volume so that when diluted the solution will be approximately N. This adjustment of acidity is of importance in matching the amount in the standards.

Procedure. Place 10 ml. of 1 per cent potassium thiocyanate solution in each of two Nessler tubes. To one add sufficient of the sample

to produce a color not greater than that developed by 5 ml. of the standard solution, and dilute to 20 ml. with water. If less an amount than 2.5 ml. is required, add the remainder of 2.5 ml. in the form of N hydrochloric acid before dilution. The N hydrochloric acid is produced with sufficient accuracy by dilution of 8.7 ml. of concentrated acid to 100 ml. To the second Nessler tube add 2.5 ml. of N hydrochloric acid or, if more than 2.5 ml. of sample solution are used, add the same volume of N hydrochloric acid. Dilute to 15 ml. with water, add standard ferric solution to match the color of the sample, and adjust the volume to 20 ml. by the usual duplication method. Multiply the standard used by a suitable factor to give the amount for the entire sample.

As a blank, add 25 drops of 50 per cent sodium hydroxide solution to 15-20 ml. of water in a 50-ml. volumetric flask. Add exactly 10 ml. of concentrated hydrochloric acid and cool the solution. Add 15 ml. of the same ferrous ammonium sulfate standard used with the sample, dilute to volume, and mix.

To each of two Nessler tubes add 10 ml. of the 1 per cent potassium thiocyanate solution. To one tube add 5 ml. of the blank solution and dilute to 25 ml. with water. To the other tube add 9.2 ml. of N hydrochloric and dilute to about 20 ml. with water. Add standard solution to approximate the color in the other tube. This will usually require about 1.5 ml. Complete the duplication as usual. Multiply the blank so obtained by 10 to equal the total blank and subtract from the value for the total sample. If the oxygen content of the gas is 0.1 per cent, the usual volume of the blank is about one-seventh that of the standard. With this content of oxygen, or more, a single blank is satisfactory for an 8-hour period to give accuracy within 2 per cent. An over-all blank is required only in work of extreme accuracy and is cumbersome. Calculate the results, correcting the volume of sample gas for temperature and pressure.

Standard. Dissolve 0.86 gram of ferric ammonium alum, $Fe_2 \cdot SO_4 \cdot (NH_4)_2SO_4 \cdot 24H_2O$, in water and dilute to 100 ml. Then dilute 10 ml. of this solution and 1.0 ml. of concentrated hydrochloric acid to 1 liter. This contains 0.01 mg. of ferric iron per ml, and each ml. is equivalent to 0.001 ml. of oxygen at normal temperature and pressure or 0.00105 ml. at 60° F. and 76.2 cm. of mercury.

Oxygen by Indigo Carmine

Dissolved oxygen is determined by the intensity of the blue developed by action on the yellow leuco-base of indigo carmine, a name for 5,5-in-

digo disulfonic acid.[5] The original method for oxygen in cultures of protozoa has been modified to apply to beer [6] and to a gas stream free from acid gases flowing at 0.5 liter per minute through the reagent solution.[7] In beer it was found that sulfur dioxide or yeast did not interfere, and good correlation was obtained with evolution and the Winkler method for dissolved oxygen. The reagent must be accurately reduced with hydrosulfite without excess being present.

Reagent. Prepare a 0.1 per cent solution of the purified, assayed indigo carmine in water and cover a portion with a 1-inch layer of neutral mineral oil. Prepare a 5 per cent solution of sodium hydrosulfite freshly for use. Fill a pipet with this solution, insert the tip below the oil, and add it dropwise to reduce the dye to a faint green color. Do this immediately before use. For use with liquids it is also convenient to calculate the relationship of the unreduced dye solution to the volume of sample to equal 1 part per million as follows:

$$\frac{(466 \times \text{volume of sample in ml.})}{(3200 \times \text{per cent assay of dye})} = X$$

Alternatively, using a 0.1 per cent solution, divide 0.1 by the calculated weight X to give the parts per million indicated in the sample per ml. of reagent.

Procedure. *Solution.* Prepare a sample bottle with a rubber stopper having one glass inlet tube run to the bottom, another just through the stopper. Have both tubes closed with rubber tubing and clamps. Flush out the sample bottle with carbon dioxide. Transfer the sample to the bottle without air absorption by flowing in from the storage container. Then an excess of reagent is to be transferred without exposure. For this quickly draw 2 or 3 drops of oil into a pipet from over the surface of the reagent, draw in leuco-base, and then draw a drop of oil into the tip to protect the reagent from the air. Take somewhat more of the leuco-base than needed so that, if a colored surface develops in emptying the pipet, it will not be introduced into the sample.

Open the clamp on the short inlet tube of the bottle and insert the pipet without admission of air. By loosening the other clamp, force the reagent into the sample until no further change in color appears. Excess will be used and the contents of the pipet are not ordinarily completely

[5] W. W. Efimoff, *Biochem. Z.* **155**, 371-5 (1925).

[6] Harold Rothchild and Irwin M. Stone, *J. Inst. Brewing* **44**, 425-31 (1938).

[7] Heinrich Macura and Günther Werner, *Die Chemie* **56**, 90-1 (1943).

transferred. Close the clamps as the pipet is withdrawn and invert the bottle to mix. Let it stand for 1 hour at 25° and read against a series of standards in bottles of the same size, prepared by addition of the unreduced reagent in known amounts to water. These bottles need not be fitted with tubes, or other precautions taken.

Gas. Pass known volumes of the gas stream through a known volume of the reagent suitably diluted with water, in a gas-wash bottle. Compare the color with that of a series of standards prepared in similar bottles by adding the unreduced reagent to water.

Oxygen by 2,4-Diaminophenol

Oxygen reacts with excess 2,4-diaminophenol in alkaline solution to give a stable blue compound of an intensity directly proportional to the oxygen concentration.[8] For samples high in oxygen, dilute with an equal volume of oxygen-free water. Sea water will give a precipitate with the reagents, which can be carried down by centrifuging. Tap water gives a faint cloudiness which will usually not interfere.

Reagent. Dissolve 1 gram of 2,4-diaminophenol dichloride in 5 ml. of 0.1 *N* hydrochloric acid. A faint red color will usually be present. Add a trace of sodium bisulfite, $NaHSO_3$, using the minimum amount which will decolorize. Mix this with 125 ml. of 95 per cent glycerol and store in a stoppered bottle. A yellow color develops in about 2 weeks which does not interfere with development of the blue but does mask it. The reagent should then be freshly prepared.

Procedure. The method is applicable either on a micro or macro basis. As described it is adapted to a 10-ml. sample. Transfer the sample to a 10-ml. glass-stoppered tube containing a glass bead, with the usual avoidance of air exposure by previous flushing with carbon dioxide. Add 0.5 ml. of the prepared reagent and 1 ml. of a solution containing 1 gram of sodium cyanide in 100 ml. of 10 per cent sodium carbonate solution. The latter should have been heated to boiling and protected from subsequent absorption of oxygen. Both should be delivered well under the surface with a minimum of air exposure. On shaking to mix, a blue color will develop in 2 minutes and be stable for about 30 min-

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[8] Moses L. Isaacs, *Sewage Works J.* 7, 435-43 (1935); F. Wellington Gilcreas, *J. Am. Water Works Assoc.* 27, 1166-77 (1935); R. Brinkmann and A. van Schreven, *Acta Brevia Neerland Physiol., Pharmacol., Microbiol.* 11, 77-8 (1941).

utes. Compare with standards or read the transmittance. The standards are prepared from water of known oxygen content, standardized by one of the well-known methods such as that of Winkler, and may be preserved by duplication with artificial standards (Vol. 1, Chapter 7).

Oxygen as Iodine

The Winkler method may be modified to liberate iodine and, instead of titrating, it is extracted with chloroform and compared colorimetrically.[9] The method will readily distinguish 1.0 from 1.2 ml. per liter of oxygen. Artificial standards such as Lovibond colors are necessary for field work. Assuming the titration value to be accurate, the method shows a standard deviation in 59 samples of ±3.26 per cent. A special pipet has been designed for use with 10-ml. samples[10] but at somewhat reduced accuracy. The quantities and concentrations of reagents must be vigorously standardized.

Procedure. Transfer the sample of water to a 100-ml. bottle without contact with air. If organic matter or nitrites are present, add sufficient 0.5 per cent potassium permanganate solution to give a permanent pink color. After 5 minutes, add 2 per cent potassium oxalate until the pink color is removed. Add 2 ml. of a reagent containing 10 grams of potassium iodide and 34 grams of sodium hydroxide per 100 ml. to the bottom of the bottle. Similarly add 0.4 ml. of 40 per cent manganous chloride solution. Shake and, when the manganous hydroxide has settled, add 0.4 ml. of concentrated sulfuric or orthophosphoric acid. In all of these avoid exposure to air. After adding acid and shaking, exposure does no harm.

Transfer 10 ml. of solution if less an amount than 4 ml. of oxygen per liter is present, or 5 ml. if above that level, to a stoppered cylinder. Add 10 ml. of chloroform and shake for 30 seconds. When the chloroform has separated, compare with a series of natural or artificial standards. If the 5-ml portion is used, multiply all results accordingly.

Standards. Take a series of water samples of varied oxygen concentration below 8 ml. per liter and carry them through the procedure. Titrate a portion of the Winkler solution obtained from each to determine its oxygen content. Either use the chloroform extracts as standards and replace after a day, or match each with an artificial standard (Vol. 1,

[9] M. L. Johnson and R. J. Whitney, *J. Exptl. Biol* 16, 56-9 (1939).

[10] R. J. Whitney, *ibid.* 15, 564-70 (1938).

As soon as the gas has attained atmospheric pressure in G, as judged by a manometer in the line of the gas flow, close cock D and disconnect the apparatus at E. Mix the solutions in A and B and pour into G by inclining the apparatus. Slowly rock the suspension of manganous hydroxide from side to side at frequent intervals during a period of one hour. At the end of an honr remove the gas in G hy attaching tbe oil pump to E, evacuate the line between D and E, and then turn D to connect G with tbe pump for a short time.

Now fill C_3 in the same way that C_1 and C_2 were filled, with 4 ml. of 20 per cent sulfuric acid and 0.5 ml. of a 1 per cent suspension of soluble starch. After allowing a short time for tbe mixture to come to room temperature, carefully fill the tnbing rbove stopcock 3 with benzene, attach a small glass Gooch crucible holder to X_3 by means of rubber tubing and fill the holder with benzene. Carefully open stopcock 3 and allow the henzene to flow onto the starch-acid mixture, until C_3 is completely filled. With stopcock 3 open, carefully open stopcock 7 so that tbe solution in C_3 can run into G, care heing taken to leave a small amount of the starch-acid mixture above stopcock 7. The oxidized and unoxidized manganous hydroxide goes into solution and the iodine liberated colors the starch. Wash the liquid around the apparatus, transfer to a comparison tube, aud compare with standards.

Standard. Place 1.5 ml. of a 10 per cent potassium iodide solution, 4 ml. of 20 per cent sulfuric acid, and 0.5 ml. of a 1 per cent suspension of soluble starch in a comparison tube and add 1 ml. of a solution of potassium permanganate containing 0.395 gram per liter. Dilute to 24 ml. The hlue color is equivalent to that produced by 0.1 mg. of oxygen. Further colors, corresponding to smaller quantities of oxygen, are prepared by using 1 ml. of more dilute permanganate solutions. Suitable standards are 0.1, 0.01, 0.001 and 0.0001 mg. of oxygen. The colors ohtained by evacuating the apparatus to a known pressure of air, and hence a known amount of oxygen, are in good agreement with tbe standard colors obtained by means of potassium permanganate. A blank test with the apparatus evacuated to 1×10^{-5} mm. should give no color.

Miscellaneous

Another method uses a mixture of dry powdered borax and a chloroderivative of hydroquinone, adurol.[13] As reagent, mix 6 parts of borax, previously dried at 50°, 1 part of adurol and 3 parts of potassium sodium

[13] L. W. Winkler, *Z. angew. Chem.* 24, 341 (1911); *ibid.* 26, 134-5 (1913).

tartrate, Rochelle salt, as the reagent. The Rochelle salt prevents precipitation of calcium and magnesium. To glass-stoppered bottles of uniform size add sufficient sample and standards to fill, then about 0.5 gram of reagent per 100 ml. of sample. Stopper and dissolve the reagent by repeated inversions. Allow to stand for 5 minutes. A reddish brown color develops. Estimate the degree of saturation by comparison of the sample with standards. The standard must be very nearly the same in color as the sample. Dilution is not satisfactory.

For such standards prepare solutions of oxygen in various degrees of saturation by shaking water with air, allowing the bubbles to rise and diluting with known volumes of water which has been boiled and cooled in the absence of air. At 20°, oxygen is soluble in distilled water to the amount of 9.17 ppm.

CHAPTER 67

PEROXIDES

In general the reactions of peroxides are those of strong oxidizing agents. This is complicated by their presence in such nonaqueous media as synthetic rubber, gasoline, drying oils, etc. These varied types of samples call for almost equally varied procedures.

One of the most sensitive reagents is ferrous ion, which is oxidized to ferric ion under suitable conditions. Ferric ion can then be determined in many ways, but the thiocyanate reagent is that most commonly used. Another is the development of the color of titanium and peroxide.

Peroxides as Ferric Thiocyanate

Not only hydrogen peroxide but related substances must be considered in this method. Magnesium, zinc, calcium and barium peroxides are converted to hydrogen peroxide in acid solution. Sodium peroxide is an alkaline solution of hydrogen peroxide. Sodium perborate or persulfate is in effect a buffered alkaline solution of hydrogen peroxide. Any of these may be determined by their oxidation of ferrous iron to ferric. The amount oxidized is estimated by addition of a thiocyanate.[1] A concentration of 1 ppm. or 0.0001 per cent of hydrogen peroxide may be determined. A large excess of thiocyanate is essential to estimation of small amounts of hydrogen peroxide.

Procedure. As reagent, dissolve 78.4 grams of ferrous ammonium sulfate, $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, and 58.3 grams of potassium thiocyanate in cold 1:4 sulfuric acid and dilute to 1 liter with the same acid. Dissolve a suitable weight, such as 10 grams, of solid sample in 175 ml. of 1:4 sulfuric acid and dilute to 200 ml. with water. If the sample is already in solution, such as a solution of hydrogen peroxide, modify to adapt as closely as possible. Divide the 200 ml. into two 100-ml. portions, one to serve as blank. Add 0.5 ml. of the reagent to the 100-ml. sample portion and compare with the standard.

[1] F. W. Horst, *Chem.-Ztg.* **45**, 572 (1921); J. S. Reichert, S. A. McNeight and H. W. Rudel, *Ind. Eng. Chem., Anal. Ed.* **11**, 194-7 (1939).

Standard. Dissolve 0.964 gram of ferric ammonium sulfate $Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$, in 80 ml. of cold 1:4 sulfuric acid, add 5.8 grams of potassium thiocyanate, and dilute to 100 ml. Each ml. of this solution is equivalent to 0.00034 gram of hydrogen peroxide. Dilute to the desired concentration with cold 1:4 sulfuric acid containing 5.8 grams of potassium thiocyanate per 100 ml.

Organic Peroxides as Ferric Thiocyanate

By working in nonaqueous solution, organic peroxides react more or less rapidly with ferrous compounds to oxidize them to ferric, after which they are determinable as ferric thiocyanate.[2] Some cracked gasolines do not react completely[3] since the residue will still react with hydriodic acid. Satisfactory correlation is obtained with hydrogen peroxide and succinyl peroxide.

Procedure. Add 5 grams of ammonium thiocyanate and 5 ml. of 1:2 sulfuric acid to 1 liter of absolute methanol. Shake this solution with finely pulverized ferrous ammonium sulfate for a few minutes to saturate it. A faint pink color is formed which must be evaluated and corrected for as a reagent blank. The color does not darken appreciably in an hour or two, but storage in an inert atmosphere is desirable if it is kept longer.

Transfer 10-ml. portions of the reagent to Nessler tubes. To one of these add 0.5 ml. of the sample, if low in peroxides, or 0.5 ml. of a solution of known concentration in absolute methanol if high in peroxides. The rate of color development varies. With butyl acetylene and 1-hexene it develops in a matter of seconds in the cold. Peroxides or diamylene react slowly and the tube should be immersed in boiling water for 4-5 minutes. Develop the maximum color in all cases. Compare the developed color with that resulting from known standards made by added 0.5 ml of standard diluted with methanol to the same volume of reagent the same day. The color fades on standing for a longer time.

Standards. Dissolve 0.05584 gram of pure iron wire in a small volume of 1:4 hydrochloric acid, avoiding loss by spattering. Evaporate to

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[2] Charles A. Young, R. R. Vogt and J. A. Nieuwland, *Ind. Eng. Chem., Anal. Ed.* 8, 198-9 (1936); Charles D. Wagner, H. Lawrence Clever, and Edward D. Peters, *Anal. Chem.* 19, 980-2 (1947).

[3] J. A. C. Yule and C. P. Wilson, Jr., *Ind. Eng. Chem.* 23, 1254-9 (1931); Charles D. Wagner, Richard H. Smith, and Edward D. Peters, *Anal. Chem.* 19, 982-4 (1947).

dryness without decomposition, take up in absolute methanol, and dilute to 1 liter. The solution contains 0.559 mg. of iron per ml. and is 0.01 *M*. From this prepare a series of dilutions in absolute methanol. A suitable range is 1, 3, 5, 10, 20, 30 and 50 ml. diluted to 100 ml. These are added at 0.5 ml. per 10 ml. of reagent, parallel to development of the sample.

Peroxides in Synthetic Rubber by Ferric Thiocyanate

A modification[4] of the previous method permits determination of combined peroxides in rubber. It is applicable to all samples soluble in hydrocarbon solvents, which necessarily prevents its application to vulcanized samples. Oxidation inhibitors do not interfere. Accuracy of 5-10 per cent is obtained and the method will go as low as 10 ppm. in the sample. The solution read should not contain over 25 ppm. The time for full development of color varies from 5 to 75 minutes. Gentle warming to facilitate development of color is feasible but not advisable.

Sample. Use a sample as homogeneous as possible and fairly dry. Milling in air may add peroxides. Dissolve about 0.60 gram in 20 grams of benzene in a container which is substantially full. Shake or tumble to dissolve, then filter if necessary. Soluble ferric iron, if present, must be separately determined with thiocyanate and a correction applied.

Procedure. As reagent dissolve 0.130 gram of potassium thiocyanate in 50 ml. of absolute ethanol and add 0.065 gram of ferrous chloride tetrahydrate. When dissolved, add sufficient of this solution to 79 ml. of chloroform to dilute to 100 ml. Acidify with 2 drops of concentrated sulfuric acid. The reagent so obtained should be substantially colorless. Potassium chloride separates and should be centrifuged out. This reagent is stable for several hours in the dark.

Add 1 volume of sample solution to 15 volumes of the reagent and read the color by measurement of transmittance. Continue to read so long as more color develops. For preparation of the standard curve dissolve known weights of ferric chloride hexahydrate in methanol and dilute to 20 times the volume with chloroform. If the color developed by the sample is too deep, cut it back with benzene.

Hydrogen Peroxide by Oxidation of Titanium Sulfate

The reaction will qualitatively detect 1 ppm. of hydrogen peroxide

[4] Richard F. Robey and Herbert K. Wiese, *Ind. Eng. Chem., Anal. Ed.* 17, 425-6 (1945).

in the absence of other oxidizing agents.[5] Parallelism with methods for determination of titanium indicates quantitative adaptability.[6] The reaction is one of formation of pertitanic acid:

$$Ti^{4+} + H_2O_2 + 2H_2O \rightarrow H_2TiO_4 + 4H^+.$$

The color develops instantly, corresponds to Beer's law, and is stable for at least 6 hours. Within limits the color is independent of the excess of reagent added. If the transmittance is measured, there is advantage in use of a band of 380-430 mμ rather than one centered at 470 mμ. The average deviation of the method is ± 0.03 provided a cell depth proportional to concentration is selected. Solutions which are too dark may be diluted.

Reagent. Digest 1 gram of anhydrous titanium dioxide with 100 ml. of concentrated sulfuric acid for 15-16 hours on a sand bath at 150°. Cool and dilute with 4 volumes of distilled water. Filter through an asbestos mat before use.

Procedure. Transfer a sample containing 0.1-3.0 mg. of hydrogen peroxide to a 100-ml. Nessler tube. Dilute to about 75 ml., add 10 ml. of the titanium sulfate reagent, and dilute to volume. Compare the intensity of yellow color with natural standards by any of the methods.

Standards. Dilute 20 ml. of 30 per cent hydrogen peroxide to 1 liter with distilled water and standardize by titration with potassium permanganate.

Peroxides in Natural or Synthetic Rubbers by Ferric Phenanthrolinate

This reagent has advantages over ferric thiocyanate for this type of reaction.[7] The complex formed with ferrous iron is stable for months and it follows Beer's law very closely. Excess reagent or acid does not affect the color. By avoiding the use of large amounts of inorganic salt

[5] Nelson Allen, *ibid.* 2, 55-6 (1930).

[6] J. S. Reichert, S. A. McNeight and H. W. Rudel, *Ind. Eng. Chem., Anal. Ed.* 11, 194-7 (1939); C. B. Allsopp, *Analyst* 66, 371 (1941); George M. Eisenberg, *Ind. Eng. Chem., Anal. Ed.* 15, 327-8 (1943); Paul Bonét Maury, *Compt. rend.* 213, 117-19 (1944).

[7] H. A. Laitinen and J. S. Nelson, *Ind. Eng. Chem., Anal. Ed.* 18, 422-5 (1946).

the solvent can be largely benzene and therefore contain more rubber. Unlike the thiocyanate, the reagent is stable to air oxidation. Ferrous iron, not converted to the complex, is oxidized if no antioxidant is present in the polymer. With antioxidant present, reduction of rubber peroxide can be incomplete. Then orthophosphoric acid must be added to lower the ferrous-ferric oxidation potential and cause complete reaction. Nitric acid prevents color due to ferric ion.

The peroxides present in natural rubber [8] are all hydroperoxides, as are also those in polyisoprene, polybutadiene, and copolymers of isoprene or butadiene with styrene, acrylonitrile, or other vinyl-type monomers. Disubstituted organic peroxides do not react. The method as given is sensitive to 10-20 ppm. of active oxygen on the solid polymer. The sensitivity can be increased to correspond to less than 1 ppm. of active oxygen, the limiting factor being the color of the polymer solution.

Procedure. As reagents prepare the following. Bubble nitrogen through a liter of methanol for 30 minutes to remove dissolved oxygen. Add 13.9 ml. of concentrated sulfuric acid and dissolve 19.605 grams of ferrous ammonium sulfate hexahydrate in this acid methanol. For long-time storage, keep in an atmosphere of nitrogen. For daily use, dilute 2 ml. of this 0.05 *N* solution to 0.002 *N* by diluting to 50 ml. with methanol, without adding more acid. Mix 1 ml. of concentrated nitric acid with 20 ml. of methanol slowly with cooling. Add 6.75 ml. of 85 per cent orthophosphoric acid to methanol and dilute to 100 ml. For use, dilute 2 ml. of this to 50 ml. with methanol. Dissolve 1 gram of polymer in nearly 100 ml. of benzene and dilute to volume. Transfer 1 ml. containing 0.01 gram of polymer to a 50-ml. volumetric flask. Add about 25-ml. of thiophene-free benzene, rinsing down the sides of the flask.

Add 1 ml. of the dilute nitric acid in methanol and 0.5 ml. of the dilute orthophosphoric acid in methanol, shaking after each addition. Add 1 ml. of the dilute ferrous solution, mix well, and let stand for 15 minutes Add 5 ml. of 0.1 per cent solution of *o*-phenanthroline in thiophene-free benzene. Dilute to volume and mix. Read the transmittance at 500-510 mμ, using the sample diluted but without reagent to obtain the zero setting. Unless the transmittance is 50-70 per cent, use only as a preliminary figure and repeat with the sample calculated to fall in that range. Read the results from the usual calibration curve constructed without sample. Thus the reading is in terms of ferrous iron.

[8] Ernest Harold Farmer and Donald A. Sutton, *J. Chem. Soc.* 1942, 139-48.

Calculate from the following **formula**:

$$\frac{(A - B) \times 8 \times 10^6}{C \times V} = \text{micrograms of active oxygen per gram of polymer} = \text{ppm. of active oxygen}$$

where

A = equivalents of ferrous iron taken
B = equivalents of ferrous iron remaining
C = concentration of polymer in grams per ml. of benzene
V = ml. of polymer solution used

Organic Peroxides by Titanium Sulfate

The oxidation to titanic sulfate, which gives a yellow color, is a convenient method of estimation.[9]

Reagent. Add 1 gram of titanium sulfate to 100 ml. of 1:4 sulfuric acid and heat until solution is complete. Dilute to 1 liter with cold water and filter.

Procedure. Mix 30 grams of fat or oil sample and 15 ml. of the reagent solution and shake for 20 minutes. Raise the temperature to 60° and again shake for 5 minutes. Centrifuge to separate the aqueous layer from fat. If necessary, extract fat with petroleum ether. Finally filter the aqueous layer to give a clear yellow solution and compare with standards previously set up. This may be by use of organic peroxides or calibration of transmittance around 400 mμ.

[9] R. Strohecker, R. Vaubel and A. Tenner, *Fette u. Seifen* 44, 246-50 (1937).

CHAPTER 68

OZONE

THE ILLUSIVE nature of ozone as something developed in the air, only available in dilute gaseous form and often accompanied by oxides of nitrogen, complicates its estimation. It is a powerful oxidizing agent. The methods for determination are limited to applications of that property by oxidation of iodide, nitrite, or fluorescein leuco base.

OZONE BY LIBERATION OF IODINE

About 0.001 mg. of ozone can be detected in air by leading the gas through a wash-bottle containing an alkaline or neutral solution of potassium iodide. The reaction is $O_3 + H_2O + 2I^- = I_2 + 2OH^- + O_2$, in which 1 molecule of ozone liberates 1 molecule of iodine. When an acid solution is used, more than 1 molecule of iodine is liberated by a molecule of ozone. Other oxidizing agents must be absent.

Procedure. Pass the gas sample through 20 ml. of a 0.01 per cent solution of potassium iodide and 0.01 per cent potassium hydroxide for sufficient time to give a clear, positive test on addition of starch solution. Record the volume of gas passing in the specified time. Rinse the contents of the wash-bottle out completely, add 1 ml. of 1 per cent boiled starch paste, and dilute to 25 ml. Compare with a series of standards similarly developed from an alkaline solution containing 0.001 mg. of iodine and 0.01 mg. of potassium iodide per ml.

Standard. Dissolve 0.1 gram of iodine, 1.0 gram of potassium iodide, and 1.0 gram of potassium hydroxide in water and dilute to 1 liter. Dilute 10 ml. to 1 liter. Each ml. contains 0.001 mg. of iodine. As standards, use varying volumes of this solution diluted to 20 ml. with a solution containing 0.01 mg. of potassium hydroxide per ml. Prepare the latter in the same way as the standard iodine solution, omitting the iodine.

Ozone by Destruction of Nitrite

The destruction of nitrite by ozone has been utilized for its determination.[1]

Procedure. Two samples of air are collected, one having been passed through a U-tube of glass beads carrying chromic acid and powdered manganese dioxide, the other chromic acid only, both at freezing temperatures to remove sulfur compounds. The manganese dioxide destroys ozone. Shake each sample with 25 ml. of 0.0002 per cent sodium nitrite in 0.004 per cent sodium hydroxide solution and 100 ml. of water per 7 liters of gas sample. The destruction of nitrite is measured as nitrate or as nitrite remaining (Chapters 59 and 60). If nitrogen pentoxide is present, it is measured as ozone, their reaction in this case being the same.

Ozone by Fluorescein

Ozone is measured by its oxidation of the leuco-base to form fluorescein.[2] Nitrous vapors, hydrogen peroxide, traces of chlorine, and small amounts of carbon dioxide do not interfere. The method is rapid, sensitive, and specific. The fluorescence is stable in alkaline solution. The reported empirical factor does not agree with experience in the author's laboratory, and therefore standardization for the conditions of use is needed.

Reagent. Dissolve 1 mg. of fluorescein in 1 ml. of 10 per cent sodium hydroxide solution. Add 10 ml. of saturated sodium hydroxide solution. Reduce by shaking with 1 gram of zinc dust until fluorescence is no longer observed, and filter.

Procedure. Add 1 drop of reagent to 10 ml. of 0 5 per cent sodium hydroxide solution in a test tube. Pass air through the solution at not over 15 liters per hour until the color matches that of a standard containing 1 part of fluorescein in 100,000,000 parts of water Calculate the ozone in the air on the basis of previous calibration with known amounts of ozone.

[1] Francis L. Usher and Basrur S. Rao, *J. Chem. Soc* T111, 799-809 (1917); G. A. Gorodetzkiĭ, *J. Applied Chem* (U.S.S.R.) 9, 353-61 (1936).

[2] M. S. Egorov, *Z. Untersuch. Lebensm.* 56, 355-61 (1928).

AUTHOR INDEX

SUBJECT INDEX